2009

EVALUATING THE EFFECTS OF ORGANIC AND CONVENTIONAL INPUTS ON SOIL CHEMICAL AND BIOLOGICAL PROPERTIES IN A FOUR-YEAR VEGETABLE ROTATION AND THE INVESTIGATION OF SOIL MICROBIAL PROPERTIES ON PLANT GENE EXPRESSION

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ABSTRACT OF DISSERTATION

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The Graduate School

University of Kentucky

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ABSTRACT OF DISSERTATION

A dissertation submitted in partial fulfillment of the
requirements for the degree of Doctor of Philosophy in the
College of Agriculture
at the University of Kentucky

By
Audrey Law

Lexington, Kentucky

Director: Dr. Mark A. Williams, Professor of Horticulture

Lexington, Kentucky

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The objective of this research was to determine the effects of conventional inputs on soil chemical and biological properties compared to organic systems in a four year vegetable rotation. Tillage and cover crops were the same in all treatments to avoid confounding factors often present in similar research. Additional experiments investigated plant gene expression in organic and conventional management systems and in soils with decreased microbial diversity. Experimental plots were prepared in the spring of 2004; four replications of three management treatments, organic, low-input and conventional, were arranged in a randomized complete block design. The rotation consisted of edamame soybean, sweet corn, fallow (pastured poultry in organic plots), and potatoes. Soil samples were taken in the spring and fall of each year, along with data for pest damage, weed control, yield and quality. Soil samples were analyzed for enzyme activity (maximum activity under substrate saturation) and basic soil chemical properties. Treatments were compared over time using 2-Way ANOVA. Multiplex terminal-restriction fragment length polymorphism (M-TRFLP) profiles of the soil microbial community were compared using Multiple Response Permutation Procedures (MRPP). Multi-way ANOVA detected significant treatment effects over time in total carbon, nitrogen, Mehlich III K, Exchangeable K and exchangeable Na (p=0.05). Many significant changes in soil properties over time could not be attributed to treatment effects. All treatments produced similar yields, indicating that successful organic production of these vegetables is possible in Kentucky. Input costs for organic were 37% higher than conventional, due to the cost of organic fertilizer. The organic system required nearly 50% more labor hours than conventional or low-input. The low-input system was the most cost effective, with 58% less input expenses than the conventional system. Microarray analysis of approximately 37,500 *Glycine max* transcripts did not show significant differences in the gene

expression between plants grown organically and conventionally, in plots with significant soil chemical and microbial differences. An experiment in progress is investigating changes in plant gene expression using real time RT-PCR in tomatoes grown in autoclaved soil and native field soil.

Key words: Agricultural sustainability, agricultural systems, organic agriculture, soil biodiversity, soil quality

Audrey Law

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Student's signature

March 17, 2009

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Date

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Dedicated to my Grandfather, “Dr.” Reverend Larry Buskirk, who inspired me with his love of learning, poetry, the outdoors, corny jokes, and of course, his family

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Chapter 1. Introduction

Technological advancements in agriculture are largely responsible for much of human progress. A steady, affordable, high quality diet improves health, extends life expectancy and fuels innovation. It is now known, however, that some of the methods used to achieve agricultural gains in recent history have had harmful effects on human health and the environment. Increasing applications of fertilizers and pesticides have lead to eutrophication, ground water contamination, unforeseen impacts on non-target organisms, and resistance of pests and weeds to chemical control methods; these problems are generating concern for future management of our vital resources and the increasing pressure for food production to meet the needs of the world's growing population (Daily et al., 1998; National Research Council (U.S.). Policy Division. Board on Sustainable Development., 1999; Brady and Weil, 2002).

Concerns about the environmental impacts and overall sustainability of agriculture are increasingly influencing how consumers, producers and researchers envision the future of food production. A rising number of health and environmentally conscious consumers are creating a demand for organically grown food, which is produced without the chemical inputs that characterize modern conventional farming (United States Department of Agriculture Economic Research Service., 2007). In an effort to meet these demands, the number of acres farmed organically in the U. S. continues to rise (Greene, 2007). This represents only a small percentage of food production in the United States, however, the larger picture of overall sustainability in agriculture continues to face many challenges. For example, there is disagreement over the role of organic agriculture in mainstream food production and how to evaluate system sustainability. Addressing these concerns requires greater understanding of the long-term effects of agricultural systems, viable alternatives to harmful practices, and the sustainable use of natural resources. In response to these needs, research in this field is expanding rapidly. The number of sustainability-themed articles published in scientific journals have been increasing annually by 15-20%

over the last decade, prompting the Proceedings of the National Academy of Sciences journal to create a new section specifically dedicated to sustainability science (Clark, 2007).

The three experiments detailed in this dissertation examine different aspects of sustainable agriculture relevant to vegetable production in the state of Kentucky and address fundamental questions regarding the evaluation of sustainable agriculture and soil resource management. In Chapter Two, three agricultural management systems are compared in a four-year vegetable rotation: conventional, low-input and organic. The impact of conventional chemical inputs on soil quality parameters was investigated, and each management system was evaluated with respect to yield, weed, pest and disease factors. Chapter Three describes experiments that focused on the effects of the soil microbial community on plant health as a means to better understand the significance of soil biodiversity in the context of agricultural sustainability.

Chapter 2. Review of Literature

Sustainability in agriculture

The term “sustainable agriculture” is increasingly included in the dialogue concerning food production around the world. While there is currently no consensus on what actions are necessary to achieve it, a working definition has been put forward by several authorities, including the United States Congress in the 1990 Farm Bill. The law states, “the term sustainable agriculture means an integrated system of plant and animal production practices having a site-specific application that will, over the long term: satisfy human food and fiber needs, enhance environmental quality and the natural resource base upon which the agricultural economy depends, make the most efficient use of nonrenewable resources and on-farm resources and integrate, where appropriate, natural biological cycles and controls, sustain the economic viability of farm operations, and, enhance the quality of life for farmers and society as a whole." (FACTA, 1990). The National Sustainable Agriculture Information Service (ATTRA) defines sustainable agriculture in similar terms, but includes additional language on the use of ecology-based strategies that enhance and maintain biodiversity, result in the recycling of plant nutrients, protect the soil, and integrate crops and livestock (Earles and Williams, 2005).

Defining sustainable agriculture is reasonably straightforward - evaluating and ultimately achieving sustainability in our farming systems remains a challenge. A basic framework for the evaluation of sustainable land management was proposed by the Food and Agriculture Organization of the United Nations (FAO) in the World Soil Resources Report in response to the challenges of maintaining stable food production for a growing world population. In this report, three types of information were described, which taken together, form the basis of determining the sustainability of a particular land use: indicators - measurements of properties that reflect environmental status or change in condition; criteria - standards that govern judgments on environmental conditions; and thresholds -

the point at which a system undergoes significant change (Smyth et al., 1993). There are limitations in the application of any type of framework for evaluating sustainability, notably the lack of a common understanding of sustainability among scholars and practitioners, difficulty in demonstrating causal links between management practices and sustainability indicators and difficulty evaluating indirect or intangible benefits of certain practices (Suvedi et al., 2003). What constitutes indicators, criteria and thresholds for the sustainability of a specific land use in a particular area is the subject of ongoing research that strives to improve understanding of how natural ecosystems work, how different actions effect them, and the possible implications of those effects on long term sustainability.

Organic Agriculture and Systems Research

Due in part to increased concerns about sustainability, organic farming has become the fastest growing segment of agriculture in the U.S., developing into a solid and likely permanent force in the market (Dimitri and Green, 2002; Greene, 2007). Organic as an industry label is now defined and regulated by the USDA. The Organic Foods Production Act (OFPA) passed by congress in 1990 required the USDA to develop national standards for organic agricultural products, leading to the formation of the National Organic Program (NOP) and the National Organic Standards Board (NOSB) which established standards on production, handling and labeling of organic goods. The Organic Materials Review Institute (OMRI) maintains a list of inputs that are allowed in certified organic operations.

The NOSB describes organic agriculture as “an ecological production management system that promotes and enhances biodiversity, biological cycles and soil biological activity. It is based on minimal use of off-farm inputs and on management practices that restore, maintain, and enhance ecological harmony.” (Kuepper and Gegner, 2004; Organic Trade Association, 2008). Organic systems are generally characterized by the copious use of soil amendments such as compost, manure, and green manure from cover crops. These practices add organic matter to the soil, restore nutrients removed by crops, and maximize

populations of beneficial organisms such as earthworms and microbes that cycle nutrients and contribute to the stabilization of soil structure. Typically, extensive crop rotations are employed to break pest and disease cycles in the soil and to promote biodiversity, which is valued in organic systems because it is thought to encourage a balance of beneficial and harmful organisms, preventing pests and diseases from flourishing (Zimmer, 2000; Kuepper et al., 2004). This principle extends to the soil microbial community, where highly active and diverse microbial populations are thought to suppress weeds and disease and improve the plant's defense response and nutrient uptake efficiency (Kremer, 1993; Kremer and Li, 2003; Kuepper et al., 2004). Many who support organic farming believe a system that encourages biodiversity and the maximization of ecological methods of plant protection is a more sustainable alternative to the use of chemical inputs in conventional farming; a common concern is that the use of chemical inputs, especially on the scale of industrial agriculture, contributes to loss of biodiversity and the degradation of natural resources. In contrast, some raise questions about the ability of organic farming to produce sufficient yield and quality necessary to maintain economical sustainability, asserting that conventional systems have the potential to yield more on less land (Trewavas, 2001). These perceptions, along with the burgeoning market for organic products, have lead to a rise in peer-reviewed research aimed at addressing the claims of both proponents and detractors of organic farming.

As research involving organic and alternative agriculture has developed over the years, it became apparent that a different approach beyond traditional reductionist experimental designs was needed. The study of sustainability in agriculture depends on the capacity to assess effects of agricultural practices on farmers, communities and the environment. The need to balance environmental quality, human needs and economic stability is often referred to as "the three pillars of sustainability": environment, community and economy (Lehtonen, 2004). Attempts to address these three factors in agriculture has led to the study of whole systems, a concept based on the idea that whole systems have qualities and characteristics apart from the sum total of their individual components, and

that understanding the whole is key to a better understanding of the parts (Savory, 1988; Ikerd, 1993). Furthermore, a systems approach is a more effective way to evaluate organic and alternative agriculture because the management strategies in these systems are designed to work together, often relying on cumulative effects of several different practices to achieve acceptable yields and levels of pest, disease and weed control (Kuepper et al., 2004).

There is variability in yield and economic assessments comparing organic and conventional, often differing depending on the crop and whether or not organic price premiums or government subsidies are accounted for. Several studies found no statistical difference in yields between organic and conventional systems across several crops, and some even found that organic yields were higher in drought years (Dobbs and Smolik, 1996; Hanson et al., 1997; Clark et al., 1999; Delate et al., 2003). A 22-year experiment growing corn and soybeans at the Rodale Institute found that in the first five years, organic corn yields were lower than conventional, but in subsequent years yielded similarly; soybean yields were consistently similar in the two treatments (Pimentel et al., 2005). Alternately, two studies comparing the yields of wheat and other cereal grains found organic yields on average were 30-50% lower than conventional (Nguyen and Haynes, 1995; Maeder et al., 2002). Most of the common plant varieties used commercially have been developed for conventional agriculture, and may not be the most robust strains due the reliance on chemical methods of pest and disease control and high fertilizer inputs (Lammerts van Bueren et al., 2008). Plant breeding efforts are seeking more vigorous strains that could be more successful in organic systems (Baresel et al., 2008; Voorrips et al., 2008; Wolfe et al., 2008),

A systems-based approach is often used by researchers to compare conventional agriculture with alternatives such as organic and biodynamic farming in terms of soil quality and biodiversity – key indicators of agricultural sustainability. A majority of studies in recent years have found that organic farms are generally characterized as having higher biodiversity than conventional farms, with respect

to birds, mammals, invertebrates and arable flora (Hole et al., 2005). There is also evidence that organic management may result in greater numbers and diversity of soil microorganisms (Shannon et al., 2002; Wu et al., 2008). Higher arbuscular mycorrhizal fungus colonization potential, ATP concentrations, and diversity of enzyme metabolic function were found in organic systems compared to conventional, as well as increased pools of stored nutrients, enzyme activity, organic matter content and microbial biomass (Clark et al., 1998; Bending et al., 2004; Cardelli et al., 2004; Monokrousos et al., 2006). In contrast, other studies found declining levels of nutrients such as nitrogen, phosphorous and potassium in organic systems over time (Eltun et al., 2002; Gosling and Shepherd, 2005).

Soil Quality

The rise in agricultural systems studies concerning soil quality and microbial properties is a reflection of the importance of soil to the understanding of agricultural sustainability - how management practices impact the soil is fundamental in evaluating the sustainability of an agricultural system. More than just a substrate for supporting root structure, the soil has it's own complex ecosystem in which microorganisms are the dominant form of life and are responsible for performing functions vital to soil productivity, such as decomposition of organic matter and the cycling of major nutrients important to plant growth (Sylvia, 1998). Brady and Weil (2002) describe soil quality as the capacity of soil to function within its ecosystem boundaries to sustain biological productivity and diversity, maintain environmental quality, and promote plant and animal health; properties that can be measured quantitatively and adequately characterize the soil's ability to perform these functions are potential indicators for soil quality.

Soil properties are heavily influenced by soil type, which is determined by the parent materials from which the soil formed and various climate conditions that effect weathering and biological influences; however, they are also affected by use and management (Larson and Pierce, 1994; Brady et al., 2002; Bossio et al., 2005; Ulrich, 2006). Research towards identifying possible soil quality indicators

has provided a basis for their use in studies that attempt to assess the relative sustainability of agricultural systems (Ndiaye et al., 2000; Bending et al., 2004; Shukla et al., 2006; Moebius et al., 2007). With increasing use of soil quality indicators by researchers come concern about their validity when the complex and dynamic nature of soil is still far from being fully understood (Sojka and Upchurch, 1999). Proponents of the soil quality concept assert that monitoring trends and changes of soil properties over time is the most practical and realistic method available for evaluating quality, while basic research towards better understanding of soil processes remains essential to improving the application of the concept (Karlen et al., 2001).

Soil chemical and physical properties are often used to give an indication of the potential productivity of the soil in the context of crop-specific nutrient requirements. Soil samples are tested for a variety of macro and micro nutrients for use in fertilizer recommendations, and pH is measured so that the soil can be adjusted to the optimal range of the crop. Beyond the seasonal needs of a specific crop, various properties can be monitored over time to determine whether fertility is decreasing, increasing, or being maintained, or if soluble salts or heavy metal contamination is building up in the soil (Bindraban et al., 2000).

Soil biological properties are gaining interest as possible indicators of soil quality because they relate to vital soil functions such as decomposition, nutrient cycling, and the formation of soil structure. It is thought that microbial activity and population dynamics may be earlier, more sensitive indicators of change in soil quality than chemical and physical soil properties (Turco et al., 1994). While it is agreed that microbial activity is imperative for sustained soil productivity, little is known about the actual soil microbial population because it is currently impossible to culture and directly study the majority of microorganisms present in the soil environment (Ward et al., 1990). Biological properties are also sensitive to weather conditions and seasonal changes, which must be taken into account when attempting to assess management effects (Bastida et al., 2008; Meier et al., 2008).

Enzyme activity is a potential soil quality indicator. Vital biochemical processes in the soil such as the decomposition of organic matter and nutrient cycling are carried out by enzymes, which are primarily produced by soil microorganisms (Ladd, 1978; Bandick and Dick, 1999; Caldwell, 2005). Soil enzymes exist as either biotic - associated with living cells in the cytoplasm, periplasm and attached to outer cell surfaces, or abionitic - excreted into soil solution by living cells or released from lysed cells, which can be stabilized by adsorption to soil colloids, remaining functional for an indeterminate amount of time (Burns, 1982; Dick, 1994). Methods of measuring soil enzyme activity are not able to distinguish between biotic and abionitic activity (Burns, 1982; Boyd and Mortland, 1990; Dick, 1994)

The ability to study soil biodiversity has been improved by technology that does not depend on culturing microorganisms in the laboratory. Techniques have been developed to analyze the genetic diversity of soil microorganisms based on PCR amplification of a variable region of the genome thought to be unique at the species level which is flanked by highly conserved regions, such as the 16S ribosomal RNA (16S rRNA) gene sequence in bacteria and Internal Transcribed Spacer (ITS) regions in fungi (Lane et al., 1985; Ward et al., 1990; Head et al., 1998; Martin and Rygiewicz, 2005). The amplified sequences can be analyzed to construct microbial community profiles; however, the technique is subject to biases that accumulate in sampling, sample processing, DNA extraction, and PCR amplification, therefore it can not accurately characterize the species diversity present in the soil (v. Wintzingerode et al., 1997; Torsvik et al., 1998).

More information is needed about soil microbial populations, particularly the role of microbial biodiversity in soil quality and productivity, to better interpret measurements of soil biological properties with respect to agricultural sustainability. There has been research suggesting that soil biodiversity could be a factor in weed and disease suppression in the soil and improved plant resistance to pest and disease (Kremer, 1993; Kremer et al., 2003; Janvier et al., 2007). Another key consideration is how biodiversity contributes to the resistance

and resilience of the soil; that is, the ability of the soil ecosystem to resist stress and recover from the loss of important soil functions in the event of major disturbances (Degens et al., 2001; Girvan et al., 2005; Brussaard et al., 2007). Continued research into how different management systems affect diversity and functionality of the soil microbial population will provide information that will contribute to the development and promotion of more sustainable practices (Doran et al., 1987; Shannon et al., 2002; Bending et al., 2004; Crecchio et al., 2004; Fließbach et al., 2007).

Summary

While efforts to increase sustainability in agriculture are widely recognized as a high priority for the security of future food production, there is less consensus on how sustainability is best evaluated. Organic agriculture has risen steadily in U.S. and around the world, in part because of its promotion as a more sustainable system that promotes biodiversity, enhances soil quality, and avoids the use of chemicals that can have negative effects on non-target organisms in the environment (Organic Trade Association, 2008). Research that investigates the sustainability of different agricultural systems has found that organic management often results in different soil biological properties, but not enough is known about the soil ecosystem to understand relationships between soil microbial diversity, soil quality, sustainability and plant health.

Chapter 3. Organic, low-input, and conventional mangement effects on soil quality, microbial communities and plant production

Introduction

Organic agriculture generally promotes soil improvement by using amendments such as compost, manure, and cover cropping. Integration with livestock is encouraged as a way to increase soil fertility and reduce off-farm inputs. In addition to a generally different approach to soil management, organic systems are also characterized by the absence of synthetic chemicals used in conventional farming. Organic standards prohibit the use of these chemicals because they often affect non-target organisms, reducing biodiversity and killing beneficial insects (Kuepper et al., 2004). Some assessments have attributed the use of pesticides at recommended rates to costs in the billions in public health, pest resistance, bird loss, increased pest pressure due to loss of beneficial insects, water contamination and other environmental damages (Pimentel, 2005).

In recent years, multiple studies comparing conventional and organic agriculture have reported differences in soil chemical properties, higher microbial activity and diversity in organically managed soils, or distinct microbial profiles between the two systems (Clark et al., 1998; Øvreås and Torsvik, 1998; Shannon et al., 2002; Bending et al., 2004; Cardelli et al., 2004; Crecchio et al., 2004; Monokrousos et al., 2006; Esperschütz et al., 2007; Fließbach et al., 2007; Wu et al., 2008). It cannot be said with certainty what is responsible for the changes seen in soil chemical, biochemical, and microbial properties seen in these studies, which compared systems with different crop rotations, tillage methods, or cover cropping, and/or compared farms from different locations with differing management histories. These all represent confounding factors that may have had an effect on soil properties, and are not necessarily reflective of practices confined to either system. While no-till, conservation tillage and cover cropping may be integrated into conventional systems, the use of most agricultural chemicals is forbidden in organic farming (Organic Trade Association, 2008).

Tillage, rotation and cover cropping are known to affect soil microbial and chemical properties (Doran, 1980; Dick, 1984; Govaerts et al., 2007; Motta et al., 2007). This raises the question: how significant is the use of conventional chemicals on soil properties compared with approved organic inputs, when everything else is the same?

Few studies evaluate effects of agro-chemicals on soil microbial communities. Tests required for approval of new pesticides and herbicides only asses their effects on functional parameters such as metabolic activity using culture dependant methods; they do not examine the effects on the diversity of the microbial community, which can change dramatically even when nitrogen and carbon metabolism are unchanged (Johnsen et al., 2001). Some studies using PCR amplification of rRNA genes from genomic DNA extracted from the soil have found differences in the microbial profile of soils treated with certain pesticides and herbicides (Engelen et al., 1998; el Fantroussi et al., 1999). Girvan et al. (2004) found that pesticides did not have an effect on soil microbial communities, but different fertilization regimens did.

There remains a need for a better understanding of how these chemicals affect the soil environment, and whether or not these effects are a major factor in the soil differences seen between organic and conventional systems found in other research. This experiment was designed to address this by measuring the soil chemical and biological properties of organic, conventional and low-input systems in four-year vegetable rotation, keeping factors such as tillage and cover crops the same. Practical and economic factors such as yield and weed, pest and disease control were also measured. This approach is a compromise between using systems as growers commonly practice them and reducing confounding factors, allowing inputs to be the main differences between treatments. The cover crops and tillage method used in this study are characteristic of organic systems, and would not typically be found on conventional farms.

Sustainability in agriculture is an issue that must be addressed to ensure continued food production for our growing population. Research in this area is

needed to explore the way ecosystems are affected by agricultural practices, better understand the biological processes involved, determine ways in which sustainability can be evaluated, and generate alternatives that ameliorate the negative impacts of modern agriculture while meeting the needs of farmers, consumers, and the environment. From a local perspective, interest in organic agriculture has been increasing in the state of Kentucky. According to recent statistics, between 1997 to 2002 Kentucky experienced an increase in the number of certified organic acreage by 13.1% (Southern Organic Resource Guide, 2005). With the advent of the tobacco buy out program in 2004 and an increased demand for local produce, more small farm owners are becoming interested in alternatives such as organic fruit and vegetable production. The University of Kentucky College of Agriculture Strategic Plan includes the promotion of sustainable farming and food systems, including in the mission statement that "the enhancement of health and well-being of people and the environment and the expansion of economic opportunity by sharing the knowledge and tools for wise, innovative uses of natural resources and development of human potential" is a priority (The Land Grand Vision: College of Agriculture Strategic Plan, 2004). This research is in keeping with both the University of Kentucky College of Agriculture's stated mission as well as working towards the goals of the USDA set forth for agricultural sustainability. The results of this study will add to the knowledge base regarding the evaluation of sustainability in agricultural systems and provide a practical and economic comparison of three different approaches to vegetable farming in KY.

Materials and methods

Experimental design

The experimental plots were located at the University of Kentucky Horticultural Research Farm in Lexington, KY. Approximately one acre of a field in fescue was mold-board plowed and disked in mid-April 2004. Twelve 12 x 18 meter plots were measured out, and treatments were assigned in a randomized

complete block design representing four replications of three treatments (Figure 2.1). Plots were separated by an 8 meter buffer zone. Sudangrass (*Sorghum vulgare var. sudanense*) was broadcast on the entire field at 39.2 kg/ha. The sudangrass was originally intended to occupy the 8 meter border area between plots; however, it was difficult to manage and did not respond well to mowing. Tall fescue (*Festuca arundinacea*) was planted in the second year and remained as the border between plots through out the experiment.

All plots were tilled in using an Imants 35 Series rotary spader (Reusel, Holland), a tillage implement designed to cause minimal soil disturbance. It has rows of rotating spade-shaped blades that loosen the soil and incorporate cover crops without major soil inversion. All treatments received the same cover crop and planting rate on years when a cover crop was planted (Table 2.1).

Description of treatments by year

A summary of inputs and treatments used in each management system is listed in Table 2.2. Due to the experimental design, in which three agricultural systems were compared on the same area of land, it was not possible to do a certified organic treatment in this research; however, the plots were managed under certified organic specifications, using established resources such as the National Sustainable Agriculture Information Service (ATTRA) for information on specific crops. The conventional system in this study is characterized by the use of current recommendations in regional extension publications for each specific crop with respect to fertilizer rates and disease, pest and weed management (Bessin et al., 2006-07; Coolong et al., 2008-09). The low-input system used half rates of fertilizers, herbicides and pesticides recommended by conventional guidelines, and included some organic practices such as soil amendments and pest/weed control strategies where applicable.

Year 1 (2004): Edamame (*Glycine max*)

All plots were spaded on June 22, 2004. The conventional plots received a pre-plant incorporated herbicide – Dual Magnum at 1.5 l/ha and Canopy at 491 ml/ha according to label instructions (Syngenta Crop Protection Inc., Greensboro NC; Dupont Crop Protection, Wilmington, DE). The low-input and organic treatments received no herbicide. The powdery mildew resistant variety BeSweet 292 (Rupp Seeds Inc., Wauseon, OH) was chosen from a list of recommended varieties in the UK Vegetable Production Guide for Commercial Growers (ID-36, 2005-06). Untreated seeds were used because certified organic seed for this variety was not available. The seed was treated with *Rhizobium japonicum* inoculant (Southern States, Lexington KY) and planted using a two row no-till planter at 112 kg/ha with 46 cm row spacing (Herbek and Bitzer, 1988). Based on soil test results for phosphorus and potassium, no additional fertilizer was required (Miles et al., 2000). A weed analysis was done once before cultivating in order to compare weed pressure between treatments. A visual estimate of percent weed control in the plots was done on a scale of 0-100%. Organic and low-input plots were then cultivated by hand using wheel hoes twice before harvest. No economically damaging disease problems were present in any of the treatments. Whole edamame plants were harvested when the majority of pods and were still bright green and the beans inside were nearly touching (Miles et al., 2000). A three meter square made of PVC was placed 1.5 meters in and three meters down from the top left corner of each plot encompassing six rows, and all plants inside the square were harvested. The pods were later separated from the plants using a wooden pod stripper. The edamame pods were sorted into quality categories: two, three, and four beans per pod, and cull (damaged, rotten, one bean/pod), and weights were recorded. In the fall, compost was added at 5.5 t/ha (Creech Thoroughbred Compost, Lexington KY) to the organic and 2.7 t/ha to the low- input plots, rates recommended by ATTRA as reasonable for large scale production. The field was disked and all plots were sown with 63.8 kg/ha of rye (*Secale cereale*) and 31.4 kg/ha of hairly vetch (*Vicia villosa*). The space in between plots was planted in fescue, replacing the sudangrass.

Year 2 (2005): Sweet Corn (*Zea mays*)

Plots were mowed before being spaded in order to facilitate incorporation of the rye/vetch cover crop. A formula was used to estimate the amount of nitrogen expected to be gained from the rye/vetch cover crop, which was determined to be 78.4 kg/ha for all plots (Sustainable Agriculture Network, 1998). The organic and low-input plots received no pre-plant fertilizer. The conventional treatment received 33.6 kg N/ha ammonium nitrate (34-0-0, Green Charger, Southern States Cooperative, Richmond, VA), broadcast, and the pre-plant incorporated herbicide Bicep II Magnum (Syngenta Crop Protection Inc., Greensboro, NC) at 6.1 l/ha, following label instructions. The low-input treatment received half the recommended rate at 3.05 l/ha, and the organic treatment received no herbicide. Lancelot (Fedco Seeds, Waterville, ME), a sugar enhanced variety, was chosen because it was included in a list of varieties recommended by the Cooperative Extension Service and had high ratings for disease resistance. The seed used was untreated, however, certified organic seed of this variety was not available. The sweet corn was planted 36" rows on June 16, 2005 using a John Deer two-row planter. After germination the seedlings were thinned in all plots to 26 cm apart. When plants were approximately one meter high they were side-dressed with fertilizer. The organic treatment received 56 kg of N/ha of 10-2-8 Nature Safe organic fertilizer (Griffin Industries Inc., Cold Spring, KY). The low-input and conventional treatments received 56 kg of N/ha ammonium nitrate. As in 2004, a visual estimate of percent weed control was done for each plot. Organic plots were cultivated with wheel hoes and hand weeding (some sudan grass was not sufficiently incorporated and had to be hand-pulled – this was not included in the labor or weed assessment); no cultivation of low-input and conventional plots was done.

Soon after the appearance of silks in developing ears, control of Corn earworm (*Helicoverpa zea*) and European corn borer (*Ostrinia nubilalis*) was begun in all plots. The organic treatment was treated using a Zea-later (Johnny's Selected Seeds, Winslow, ME), a device used to inject a mixture of vegetable oil (Golden Pest Spray Oil, Stoller Enterprises Inc, Houston, TX) and *Bacillus thuringiensis*

subspecies *kurstaki* (Bt) (Dipel DF, Valent U.S.A. Corp. Agricultural Products, Walnut Creek, CA) into silks as a barrier and toxin to corn ear worm caterpillars (Hazzard and Westgate, 2004). The Bt was applied 5 days after approximately 50% of the corn was showing silk at a rate of 0.6 kg/ha, according to recommendations of the Zea-later manufacturer. The window for effective control of this method is very short – earlier application can interfere with proper pollination and later application will allow more corn ear worms to enter the ear and cause damage. The conventional and low-input corn was sprayed beginning with the first appearance of silk, three times over a two week period with Pounce 3.2 EC (Winfield Solutions LLC, St. Paul, MN) at 561 ml/ha and 280 ml/ha, respectively.

Sweet corn ears were harvested from two 3 x 3 m sections in each plot. The two sections were determined by counting in four rows in from the top left and bottom right of each end of the plot, and going in 4.6 m from the edge of the plot to mark the staring point of the 3 x 3 m section. Several parameters for the evaluation of plant growth and yield quality were measured. Ten plants in each harvested section were measured from the ground to the top leaf collar. The total weight and number of un-shucked ears were recorded and ten ears from each harvested section were randomly selected and shucked for quality assessment; the weight, length and width of shucked ears, and number of insect damage of ears were recorded. Following harvest and soil sampling, plots were disked and a cover crop of annual rye grass and white clover were sown as a summer cover for the fallow year in 2006.

Year 3 (2006): Fallow / Pastured Poultry

Four 1.5 x 1.5 meter pastured poultry pens were constructed out of 2.5 cm PVC pipe and poultry wire according to the design specifications (Dean Hunt/JC Designs) (Figure 2.2). A tarpaulin was used to cover half of the pen to provide shade and protection from weather. Twenty liter watering devices and feed trays made from 12.5 cm PVC pipe were placed in each pen. The summer cover of annual rye grass and white clover was meant to be forage material for the

chickens. After mowing, however, the annual rye was not sufficient as forage and the clover was slow to become established. A mixture of oats and soybean was drilled into the plots as additional forage three weeks before the chicks arrived. Stocking rate, feed, and general care for the poultry was based on recommendations from ATTRA publications and the book Pastured Poultry *Profits* by Joel Salatin, a farmer widely regarded as an expert in pastured poultry (Salatin, 1993; Fanatico, 2006). Local pastured poultry producer Mac Stone of Elmwood Farms (Georgetown, KY) was a resource for advice and supplied the organic poultry feed (Dale Filburn Farms, W. Alexandria, OH). Darrell Slone, manager of the UK Horticultural Research farm and experienced poultry producer, as well as faculty members specializing in poultry in the Department of Animal Sciences were consulted through out the process. Sixty-four three-week old chicks were obtained from a local poultry supplier (Shadylane Poultry, Inc., Winchester, KY). Sixteen chicks were placed in each of the four pens on the organic plots. The pens were moved once a day to fresh ground. The size of the pens is determined so that at the end of six weeks, when the chickens reach processing age, the pens will have been moved across the entire area of the plot. The chickens were fed 0.11 kg of feed per bird every day, and given fresh water. Flexible, movable electric poultry fence (Premier1Supplies, Washington, IA) was placed around each pen as protection from predators. At the end of six weeks, the chickens had been moved over the entire area of the plots; at this time the chickens were killed, processed and stored in an ice bath until they could be frozen. The processing took place at the University of Kentucky Horticulture Research farm under with the help and supervision of Darrell Slone, who provided the proper equipment.

Year 4 (2007): Dark Red Norland Potatoes (*Solanum tuberosum*)

Plots were spaded on May 21 of 2007. On July 1 and 2, Nature Safe (10-2-8) organic fertilizer was applied to organic plots at a rate of 112 kg N/ha. On both conventional and low-input plots, ammonium nitrate (34-0-0, Green Charger, Southern States Cooperative, Richmond, VA) was broadcast at a rate of 112 kg N/ha, as well as potassium sulfate at a rate of 22.4 kg K/ha. All plots were disked

after application of fertilizer and 12 furrows 91.5 cm apart were made using a Cub tractor with cultivating attachments. Dark Red Norland seed potatoes were placed 25 cm apart and furrows were closed with disk attachments on the Cub, forming hills for each row. Before closing furrows in the conventional treatments, Admire 2F (Bayer CropScience, Monheim am Rhein, Germany) was applied with a back-pack sprayer at a rate of 416 ml/ha as control for Colorado Potato Beetle (*Leptinotarsa decemlineata*) (CPB). Drip irrigation was installed along all rows. After three weeks, all plots were cultivated between rows with the Cub and a spade attachment was placed to scrape a thin layer from the tops of the hills to aid potato shoot emergence. The plots were cultivated a total of three times using disks to throw soil onto the hills to control weeds in the rows; this was the only weed control treatment for all plots. After emergence and establishment of stand (about five weeks), the first spray for Early blight control (*Alternaria solani*) was applied. Conventional treatments received a foliar spray of Quadris Opti (Syngenta Inc., Greensboro, NC) at a rate of 1.9 l/ha; low-input treatments received half this rate. Organic treatments were sprayed with copper sulfate at a rate of 1.2 kg/ha. Plants were sprayed for insect control after observing flea beetle (Family: Chrysomelidae) damage and the presence of CPB larvae at more than two per plant in some areas (Rowel, 2006). The conventional plots did not receive further treatment due to maximum application of insecticide at planting, according to product label. Low-input plots received two applications of Pounce 3.2 EC at the lowest recommended rate, 281 ml/ha. Organic plots were sprayed once with Pyganic (McLaughlin Gormley King Co., Golden Valley MN) at a rate of 1.2 l/ha , then twice more at the same rate but with a back pack sprayer, targeting only a few heavily affected areas. One week before harvest, potato vines were killed in conventional and low-input plots by application of the chemical desiccant Reward (Diquat) (Syngenta Crop Protection Inc., Greensboro, NC) at 2.3 l/ha, and mowing in organic plots. Mowing did not achieve complete kill of vines, therefore it was necessary to use a string trimmer to kill those remaining. Plots were harvested using a mechanical potato digger, and separated into size categories "A" (> 5.75 cm), "B" (4.5 – 5.75 cm), and cull (Jones and Back, 2003).

Soil sampling

Soil sampling was done twice each year, prior to planting and application of treatments in the spring and after harvest in the fall. Using a 3/4" soil probe, a composite sample from each plot was obtained by taking 15 cores each of depths 0-7 and 7-15 cm. Samples were placed in labeled plastic zip-lock bags and kept on ice. In the laboratory, samples were mixed inside the bags by hand (with gloves), and a sub-sample was placed into sterile plastic 50 ml conical tubes and put in -80˚C for 16S rRNA analysis. The composite samples were then passed through a 2mm sieve and stored at 4˚ C. A sub-sample was placed in plastic lined paper bags and left to air dry for analysis of soil chemical properties. Enzymatic assays were completed within two weeks of sampling.

Soil chemical properties

Sieved, air dried soil sample composites from each plot were submitted to the Regulatory Services facility at the University of Kentucky for analysis of routine soil chemical properties where the analyses were performed. The following describes the methods used by this laboratory.

pH (Soil *et al.*, 2000) – soil was oven-dried at 38˚C and ground to pass a 2mm screen; 10 ml of water was added to 10 cm^3 of soil, then the slurry was stirred with a glass rod and allowed to sit for at least 15 minutes but not more than 2 hours before pH was measured with a glass electrode.

Phosphorous, potassium, calcium, magnesium (Soil and Plant Analysis Council 2000a; Soil and Plant Analysis Council 2000c) - 20 ml of Mehlich III extract (0.2 N acetic acid, 0.25 N NH_4NO_3, 0.015 N NH_4F, 0.013 N HNO_3, and 0.001 N EDTA) was added to 2 cm^3 soil, shaken for five minutes and immediately filtered through #2 Whatman filter paper; the filtrate was analyzed using ICP (inductively coupled plasma spectroscopy). The results are reported as kg/ha, assuming a hectare of soil equals 22,407,463 kg and the density of air-dried soil is 1g/cm^3.

Total Carbon (Nelson and Sommers, 1982) – soil was oven dried at 38˚ C and ground to pass a 2 mm screen and 0.5 g weighed into porcelain boats which

were analyzed in a dry combustion instrument (LECO or Elementar). The % carbon in the soil was measured and reported as % weight of air dried soil.

Cation exchange capacity, bases and base saturation (Soil and Plant Analysis Council, 2000b) – 10 grams of oven dried soil, ground to pass a 2mm mesh screen, was mixed with 25 ml of 1 N ammonium acetate solution and left overnight to ensure complete saturation of sites with ammonium. The sample was then vacuum filtered in a Buchner funnel through Whatman 42 filter paper and the filtrate was brought to a volume of 100 ml and analyzed for calcium, magnesium, potassium and sodium by ICP. The bases are reported as cmol kg^{-1}. Cation exchange capacity (CEC) was determined by the leaching of ammonium saturated soil in the Buchner funnel by 10 % NaCl at pH 3 in 35 – 45 ml increments. The leachate was brought to a volume of 250 ml with 10 % NaCl and then diluted 10 fold with water. One ml of concentrated NaOH was added to convert ammonium to ammonia, which was measured using an ammonium ion-selective electrode. The equivalents of ammonium in solution were converted to CEC of soil in units of cmol kg^{-1}. Base saturation was determined as (total bases / CEC) x 100 and reported as a percentage.

Total Nitrogen (Bremner, 1996) – 0.5 g of oven dried soil, ground to pass a 2mm screen, was weighed in porcelain boats and injected into a LECO combustion unit. The % N in the sample is determined by the measuring of N_2 gas emitted upon combustion. Nitrogen is reported in units of kg/ha assuming 22,407,463 kg of soil in a hectare.

Water holding capactiy (Topp et al., 1993)– the amount of water held by oven dried, sieved soil under 0.33 atm of pressure (field capacity) minus the amount held at 15 atm (wilting point) determined the water holding capacity, and was reported as % water in soil on an oven dried basis.

Soil enzyme analysis

Soil samples used in analysis of enzyme activity were kept at 4˚C for no longer than two weeks before analysis. Alkaline phosphatase, sulfatase, β-galactosidase and cellulase activity was determined using a microplate

fluorimetric assay as maximum activity of an enzyme under substrate saturation (Marx *et al.*, 2000). The assay was conducted according to the published protocol with the exception of the solvent used in making stock solutions of the substrates. Dimethyl sulfoxide (DMSO) was used in place of ethylene glycol monomethylether (methylcellosolve) as per a suggestion by the authors when contacted about problems with substrate solubility in stock solutions. The assay is based on the use of the fluorescent compound 4-methylumbelliferone, which is released upon hydrolysis of the substrate analogs. The following substrates were used in this assay: 4-MUB-phosphate, 4-MUB-sulfate, 4-MUB-β-D-galactoside, 4-MUB-β-D-cellobioside (Sigma Aldrich Co. Ltd, St. Louis, MO). Maximum activity under substrate saturation was measured as the increase of fluorescence detected by a computerized microplate fluorometer (1420 Victor^2 multi-label counter, Perkin Elmer, Waltham, MA), measured every minute for 35 minutes. Samples were measured in duplicate; each well of the microplate contained a 200 µl reaction mixture consisting of the 100 µl substrate, 80 µl 0.1 M MES buffer (2-[*N*-Morpholino]ethanesulfonic acid,pH 6.1), and 20 µl of a soil slurry made from one gram of field moist soil added to 100 ml of sterile de-ionized water and sonicated at 50 J sec^{-1} for two minutes. Standards were included on the same microplate for each soil being analyzed, as well as a control for each enzyme using sterile water in place of soil. The standards consisted of 20 µl soil slurry for each sample, 0-140 pmol MUB, and 0.1 M MES buffer to a volume of 200 µl. Figure 2.3 illustrates the microplate set up used including concentrations of all reagents. The raw data generated by the plate reader included the time each data point was recorded (hh:mm:ss) and a numerical value of detected fluorescence. Because the plate reader measured each well one at a time, the total time taken for the assay from the first data point to the last was one hour and 17 minutes, or 4219.06 seconds. A linear regression analysis determined the slope of the increase in fluorescence over time in seconds. Only slopes significant at the 0.05 level of probability were used in further calculations; non-significant or negative slopes were given a value of zero. The equation used to calculate enzyme activity as nmol MUB min^{-1} g dw soil^{-1} is illustrated in Figure 2.4.

L-arginine deaminase activity was measured as NH_4 released after the addition of 2 ml 11.5 M L-arginine solution to 5 grams of field moist soil (in duplicate) and incubation at 37˚C for three hours (Kandler, 1995). Control samples are prepared in the same way, except immediately placed in -20˚C instead of incubation. Ammonia was extracted from incubated samples by adding 18 ml 2M KCl, shaking horizontally on low speed for one hour. Samples were filtered through #4 Whatman filter paper and the filtrate was analyzed in a microplate colorimetric method using phenol and hypochlorite in alkaline solution. A plate reader was used to determine ppm NH_4. The control sample was analyzed in the same way, and the ppm NH_4 present in the control was subtracted from the incubated sample. The amount of NH_4 present in the control was also used to compare levels of mineral nitrogen between treatments. Gravimetric water content was used to estimate the dry weight of each soil sample to calculate ppm NH_4 g dw $soil^{-1}$ $hour^{-1}$. The control samples were also used to estimate mineral nitrogen present in soil samples in the form of NH_4.

Soil microbial community profile

Sub-samples of each soil composite were frozen at -80° C in sterile 50 ml polystyrene tubes as soon as the samples were brought from the field to the lab and before they were sieved. Composite samples were mixed inside their plastic zip-lock storage bags by hand, changing gloves between each sample, to avoid cross-contamination of samples with a sieve. Multiplex restriction fragment length polymorphism was performed (Singh and Thomas, 2006). Extraction of genomic DNA from soil samples was carried out using the MOBIO UltraClean Soil DNA Isolation kit (Carlsbad, CA). A multiplex PCR reaction was performed on the extracted DNA using fluorescently labeled primers (Table 3). The PCR reaction mixture contained 2µl $MgCl^2$ (2mM), 5 µl buffer (1x), 2 µl Accuprime Taq DNA polymerase (Invitrogen Corp., Carlsbad, CA), 10 pmol of bacterial primers and 20 pmol of fungal primers (Applied Biosystems, Foster City, CA), 250 µM each deoxynucleoside triphosphate, and 1 µl ultra pure bovine albumin serum (Fisher Scientific, Hanover Park, IL). Electrophoresis on a 1% agarose gel was

used to confirm amplification. PCR products were purified using Ultraclean PCR Clean-up kit (MOBIO Laboratories, Inc., Carlsbad, CA), and quantified in ng/µl using a nanocell with a 0.2 mm pathlength (Thermo Scientific, Waltham, MA). A restriction digest was performed with enzyme Hha I (Promega, Madison, WI), in a reaction containing 500 ng of the purified PCR products, 1 X buffer, 0.1 µg μl^{-1} acetylated BSA, 20 U of restriction enzyme and sterile de-ionized water for a final volume of 20 µl. A mock digest containing DNA from one of the samples and no enzyme was conducted as a control. The reactions were incubated at 37° C for three hours followed by deactivation at 95° for 10 minutes. The digest fragments were purified using QiaQuick Nucleotide Removal Kit (Qiagen Sciences Inc., Germantown, MD) to remove salts that could interfere with optimal analysis of fragments (Grüntzig et al., 2002). Purified, digested samples were analyzed in duplicate; 1 µl was mixed with 0.3 µl LIZ-GS500 internal size standard and 12 µl Hi-Di formamide (reagents acquired from Applied Biosystems, Foster City, CA) and denatured for at 95°C for 5 minutes followed by 4°C for 5 minutes before analysis an ABI 3130 genetic analyzer (Applied Biosystems, Foster City, CA). GeneMapper software (version 4.0, ABI, Foster City, CA) was used to create profiles for each dye set representing bacteria, rhizobacteria and fungi. T-RFs were quantified using the advanced mode and second-order algorithm, considering only peaks between 50 and 500 bp in order to to exclude T-RFs that are a result of primer-diamers and remain in the range of the internal size standard (Singh et al., 2006). A manual review of each profile was conducted to ensure that the binning of peaks was consistent across all profiles and to remove allele calls with peak heights less than 100. Relative abundance of T-RFs was calculated by dividing each individual peak height by the total of all peak heights in the profile and those that were less than 5% of the total were removed; this was done to minimize the effect of differing amounts of DNA analyzed (Singh et al., 2006).

Data Analysis

Distributions of all data sets were checked for normalcy by running a Shapiro-Wilks goodness of fit test on calculated residuals. Yield, quality and weed control data was analyzed using a one-way ANOVA and Tukey HSD means comparisons. Soil chemical and enzyme data were analyzed using a multi-way ANOVA with the effects of treatment, block, time, and treatment x time. Significant treatment effects were compared with a least squares means (LSMeans) Tukeys HSD test. Principal components analysis (PCA) was used to explore soil chemical and enzyme activity data for patterns or trends that were not apparent using ANOVA. PCA is a multivariate statistical method of ordination that identifies sources of variability as well as relationships between measurements; scatter graphs of PC scores for each plot can be used to reveal trends that would not be apparent in using raw data (Townend, 2002). The first four principle components of each data set were used to create scatter graphs of each PC combination, which were used to look for groupings in the treatments. Where groupings were found, the loadings for each variable, or eigenvectors, were examined for the sources of greatest variability; the scores are on a scale of 0 – (+/-)1, the closer the score is to (+/-) 1, the more influence on the variability on that particular principle component. Few of the eigenvectors were above 0.5, therefore, scores of 0.30 and above were considered for further examination. Distribution analysis, one-way ANOVA, mulit-way ANOVA and PCA tests were performed using the software JMP 7.0 (SAS Institute Inc., Cary, NC).

Statistical analysis of the multiplex T-RFLP profiles was performed using PC-ORD 5 (MjM Software, Gleneden Beach, OR), software designed specifically for the multivariate analysis of ecological data. Whittaker's three measures of diversity, alpha, beta and gamma were applied to each TRF profile using relative abundance values (Whittaker, 1972). Alpha diversity is a measure of species richness, or total number of species in each sample unit. Gamma diversity is the overall or "landscape" diversity in a collection of sample units. Beta diversity is a measure of compositional heterogeneity for each sample unit, and is calculated by dividing gamma diversity by alpha diversity; this measure is useful in

determining the effectiveness of multivariate analysis such as ordination, which can be effected by a high level of heterogeneity in the data set (McCune and Grace, 2002).

Two methods for visualizing possible groups and trends in the data, hierarchical clustering and Non-metric Multidimensional Scaling (NMS), were used as well as Multi-Response Permutation Procedures (MRPP), a non-parametric method of testing the hypothesis of no difference between two or more groups. These techniques are considered appropriate for ecological community analysis and have been used in published studies with T-RFLP data (McCune et al., 2002; Rich and Myrold, 2004; Noll et al., 2008; Peterson et al., 2008). Both binary data (presence/absence calls indicated by either a 1 or a 0) and relative abundance data were used for each analysis. Rare T-RFs (present in less than three samples units) were deleted in order to reduce noise in the data set (complete profiles were used for diversity calculations) (McCune et al., 2002). Outlier analysis was performed for both Euclidean and Sørenson distance measures, and analyses were performed with and without strong outliers (>2.5 standard deviations) in order to gauge their effect and determine if their removal was warranted.

Hierarchical clustering analysis using Euclidean distance and Ward's method of linkage as well as Sørenson distance with flexible Beta linkage was performed and a dendrogram depicting the results was created. The two distance measures were compared to determine which performed better in grouping the data, with respect to the amount of chaining (additions of single sample units into groups) and interpretability. The NMS was performed using the Sørensen distance measure, as is recommended for the analysis of community data with this technique (McCune et al., 2002; Culman et al., 2008). NMS was carried out using the auto-pilot mode with "medium" thoroughness, which specifies: maximum number of iterations = 200, instability criterion = 0.0001, starting number of axes = 4, number of real runs = 50, number of randomized runs = 50. A final solution is recommended that represents the lowest final amount of stress, or lack of fit; using randomized versions of the data set, a Monte Carlo test is

performed to determine whether the stress value of the final solution is significantly less than what could be obtained by chance. If an acceptable solution was obtained, the analysis was run again using the parameters suggested in the results of the auto-pilot mode, with two or three dimensions.

Multi-response Permutation Procedures (MRPP) with Sørenson distance and rank transformation was used to test differences in the profiles over time, and MRPP with a blocking factor (MRBP) was used with median alignment within blocks (PC-Ord requires the use of Euclidian distance when using a blocking factor) to test for differences in treatment groups for each year (Mielke and Berry, 2001; McCune et al., 2002). The test statistic T expresses the amount of separation between groups - the more negative the number, the larger the difference – and is given a *p*-value for significance. The A-statistic describes the effect size, or heterogeneity within groups compared to what would be expected by chance; for community data, A values are typically less than 0.3 (A = 1 when all items are identical within groups) (McCune et al., 2002).

Economic Assessment

Purchased applied inputs on a per/hectare basis were calculated for each year, including vegetable and cover crop seed cost. Total labor hours for the activities associated with weed, pest and disease control were recorded. Pastured poultry was not included in the inputs to the organic system. The cost of set up, including the electric fence, materials to build the pens, feed, chicks, and other supplies was close to $2000. While most of that figure would represent a one time cost, it would take several years making the maximum profit for pastured poultry to break even on that investment, if a fallow vegetable rotation was the only use of that equipment. There is no reason why this would be the case on an actual farm – the purpose of animal integration on vegetable plots is to use what is already available. In essence, investing in all the equipment for pastured poultry in order to have one cycle of birds per year on a fallow vegetable plot would not be economically feasible. It would only make sense for a farmer who already has a pastured poultry business, in which many cycles of chickens were

being raised on other parts of the farm. Therefore, the input costs associated with pastured poultry in this study were assumed to be separate from the input cost associated with actual vegetable production.

Results

Yield, Pest, Disease and Weed Analysis

2004: Yield and quality of edamame soybean did not differ among treatments, nor was quality categories of beans per pod and unmarketable pods (Figures 2.5-2.8). Total yield was in the range of 5500 – 6000 kg/ha. Stands of soybean were visually the same between treatments, and no major diseases were present. The conventional treatment, which received a full rate of pre-plant herbicide, had significantly better weed control (Figure 2.9).

2005: Sweet corn yield as kg/ha and ears/hectare was not significantly different between treatment groups (Figure 2.10, 2.11). Yields were in the range of 10 – 15,000 kg/ha. In the quality assessment of ten randomly selected, shucked ears, the organic ears were slightly shorter (by about 4 cm) and weighed less (by 0.2-0.5 g); the treatments did not differ significantly in width or number of insect damaged ears, which was greater than 50% in all treatments (Figures 2.12-2.15). The organic treatment had significantly shorter plant heights, on average about 4-6 cm shorter than the conventional and low-input, respectively (Figure 2.16). Weed control was not significantly different between treatment plots (Figure 2.17).

2006: Pastured Poultry. In the course of six weeks, the chickens had a mortality rate of approximately 50% due to illness caused by coccidiosis (*Eimeria* sp.) and bronchopneumonia caused by bacterial infections of numerous *Pasturella* species*., Gallibacteriaum anatis bv. heamolytica* and *E. coli* (as determined by the University of Kentucky Livestock Disease Diagnosis Center). The chickens were treated with the antibiotic terramycin (Southern States) according to the advice of poultry specialist faculty in the Department of Animal Sciences, but high

mortality and poor growth remained a problem. At the end of six weeks, although the mortality rate was highly undesirable, it is believed that the objective of the treatment was achieved, that is to have poultry forage and leave their manure over the area of the plot. Soil chemical and physical properties were not statistically different in the fall of 2006, but differences in the spring of 2007 were greater than any other year. Soils results are discussed further in the following section.

2007: In the final year, potatoes grown did not have significant differences in total yield, yield of size categories A (large) or B (smaller, new-potato size), or culls (Figures 2.18, 2.19).

Soil Chemical Properties

Mehlich III extracted phosphorous at 0-7 cm soil depth had significant treatment effects, with all treatments significantly increasing over time (Figure 2.20). Phosphorous in the conventional plots started out higher than the other treatments at the beginning of the study, so the significant treatment effect is likely an artifact of field variability. All three treatments increased over time. A similar pattern occurred in the 7-15 cm depth, with the conventional treatment starting out at higher levels, but with no significant change over time (Figure 2.21). The final sampling date, in the fall of 2007, shows a sharp increase of about 60-75 kg/ha in the both the organic and low-input treatments.

Mehlich III extracted potassium at 0-7 cm had no over all treatment effects, but did show a significant treatment x time interaction (Figure 2.22). The treatments have similar levels until the spring of 2007, when the organic and low-input treatments increase about 300 kg K/ha before decreasing the fall of 2007. The 7-15 cm depth shows a very similar result, but potassium levels in the organic and low-input treatments do not decrease in the fall of 2007 as the 0-7 cm depth did (Figure 2.23). The conventional treatment increases slightly as well in the spring and fall of 2007. Overall, potassium levels increased over time in all treatments for both depths.

Mehlich III extracted calcium at 0-7 cm had a significant treatment effect, with the low-input and organic treatments having higher levels than the conventional treatment (Figure 2.24). The graph shows the conventional treatment having consistently lower calcium levels from the spring of 2005. There are no treatment effects in the 7-15 cm depth. Both depths had significant time effects, with all treatments increasing over time.

Mehlich III extracted magnesium at 0-7 cm had a significant treatment effect, with organic and low-input treatments significantly higher than the conventional (Figure 2.26). The 7-15 cm depth shows the same result (Figure 2.27). Both depths had a significant time effect, with levels increasing in all treatments from the spring of 2005.

The pH at 0-7 cm had a treatment effect showing organic and low-input treatments higher (slightly above a pH of 6) than the conventional (slightly below a pH of 6) (Figure 2.28). The 7-15 cm depth had no treatment or time effects (Figure. 29).

Treatment, time and treatment x time effects were highly significant for % total carbon at the 0-7 cm depth (Figure 2.30). All treatments increased over time, with organic increasing the most, followed by low-input and conventional. In the 7-15 cm depth, there was no treatment effect; however there was a treatment x time effect (Figure 2.31). All treatments increased in the fall of 2007, with the highest levels in the organic and low-input treatments. While significant, total means and means at each sampling point remain very close, with differences between a tenth and hundredth of a point, around 2% at 0-7 cm and 1.8% at 7-15 cm.

Treatment, time and treatment x time effects were highly significant for total nitrogen at the 0-7 cm depth (Figure 2.32). A steady increase over time occurred in all treatments starting in the spring of 2005, with organic about 500-650 kg N/ha higher than the low-input or conventional treatments. In the 7-15 cm depth,

there was no treatment effect, but there was a treatment x time effect (Figure 2.33). As in the 0-7 cm depth, an increase in total nitrogen over time occurred from the spring of 2005. All treatments remained close together until the fall of 2007, when the organic treatment increased around 600 kg N/ha above the low-input and conventional treatments.

Soluble salts at 0-7 cm did not have a treatment effect, but levels did increase over time, particularly in the fall of 2007, when all treatments increased by at least 0.1 dS m^{-1} (Figure 2.34). The 7-15 cm depth had a similar result; however there is also a sharp rise in the fall of 2005 for both the conventional and low-input, and to a lesser extent organic treatments (Figure 2.35).

The CEC at 0-7 cm decreased over time in all treatments, with most of the decline happening in the first year, from the spring of 2004 to the spring of 2005 (Figure. 2.36). After the spring of 2005, the CEC increases each year in fall, and decreases in the spring, within a range of 14–17 cmol/kg. The organic treatment is significantly higher than the conventional. The 7-15 cm depth had no treatment effect, but did have a significant time effect, with all treatments decreasing over time in a similar pattern to the 0-7 cm depth, fluctuating between 14 and 16 cmol/kg (Figure 2.37).

There were no treatment effects in % base saturation in either the 0-7 or 7-15 cm depths (Figure 2.38, 2.39). Both depths did show a time effect, with all treatments generally increasing over time, from approximately 40% to 60%.

Exchangeable potassium increased over time for all treatments, and the organic treatment was significantly higher than the conventional; the spring and fall of 2007 show the most difference between treatments (Figure 2.40). The 7-15 cm depth had no treatment effect, but time and treatment x time effects were significant (Figure 2.41). Most of the means fluctuate between 0.5 and 0.7 until the spring and fall of 2007, when the organic and low-input increased to approximately 0.8-0.9 cmol/kg and the conventional stayed around 0.6 cmol/kg.

Exchangeable calcium had no significant treatment or time effects at the 0-7 or 7-15 cm depth, with most data points between 6-8 cmol/kg (Figure 2.42, 2.43). In both depths, exchangeable calcium decreased for all treatments in the spring of 2005.

Exchangeable magnesium was significantly higher in the organic treatment than the conventional in both the 0-7 and 7-15 cm depths (Figure 2.44, 2.45). Both depths increased in exchangeable magnesium over time, but the 7-15 cm depth dropped sharply in the fall of 2006 before increasing back to previous levels.

Exchangeable sodium had a significant treatment x time effect at the 0-7 cm depth (Figure 2.46). All treatments increased in the spring of 2007, then decreased again in the fall, but the organic treatment had the highest levels of sodium during this time frame. There is a treatment effect in the 7-15 cm depth, with the organic treatment significantly higher than the conventional (Figure 2.47). Sodium levels increased for both organic and low-input treatments in the spring and fall of 2007, while the conventional treatment decreased at nearly all sampling times.

Water holding capacity had no significant effects at either the 0-7 or 7-15 cm depths (Figure 2.48, 2.49). The data for both depths had a similar pattern over time, in which the WHC decreased initially after the first measurement in the spring of 2005, increased in the spring of 2006, decreased in both the fall of 2006 and spring of 2007, and increased from approximately 10 to 21 cmol kg^{-1} in the fall of 2007.

Alkaline phosphatase activity had no significant treatment or time effects at either the 0-7 or 7-15 cm depths (Figure 2.50, 2.51). At both depths the data followed a similar pattern, with an increase in activity in the fall of 2007.

Sulfatase activity had significant time effects at both 0-7 and 7-15 cm depths (Figure 2.52, 2.53). The general trend was towards an increase over time, with a decrease in activity levels in the fall of 2005 and the spring of 2007.

β-D-Galactosidase activity had no significant effects at either 0-7 or 7-15 cm depths (Figure 2.54, 2.55). The treatments generally followed the same pattern of fluctuations, with an increase in activity in the spring of 2005 and in the fall of 2006.

Cellulase activity increased significantly over time at the 0-7 cm depth (Figure 2.56). There was no significant treatment or time effects at the 7-15 cm depth (Figure 2.57). In the fall of 2007, all treatments increased in activity from a previous low point in the spring of 2007.

L-arginine deaminase activity had no significant effects at either the 0-7 or 7-15 cm depths (Figure 2.58, 2.59). The data for all treatments followed the same general pattern, with a drop in activity in the spring of 2006 and an increase in activity in the fall of 2007 at both depths.

Mineral nitrogen as NH_4 (data derived from the L-arginine deaminase control) was not significantly different according to treatment, but did increase over time. Both depths followed a nearly identical pattern. Slight increases from about 2 mg kg^{-1} NH4 g^{-1} dry weight soil in 2004 was followed by a rather large increase after the fall of 2005, near 12 mg kg-1 in the spring of 2007, and decreasing to around 6-8 mg kg^{-1} in the fall of 2007.

Correlations between all soil variables are shown in Tables 2.4 (0-7 cm) and 2.5 (7-15 cm). Due to the high number of significant correlations, only those with r^2 values greater than 0.5 were considered for these results. At both depths, phosphorous correlated negatively with pH. Mehlich III extracted K correlated positively with Mehlich III Mg, total C, soluble salts, and exchangeable K at the 0-7 cm depth, and with exchangeable K at 7-15 cm depth. At 0-7 cm, pH correlated

positively with Mehlich III Ca, % BS, and exchangeable K, and at 7-15 cm with Mehlich III Ca, % BS, exchangeable Ca and phosphatase activity. Mehlich III Mg at 0-7 cm correlated positively with Mehlich III Ca, total C, total N, soluble salts, % BS, exchangeable K and exchangeable Mg. At 7-15 cm, Mehlich III Mg correlated positively to Mehlich III Ca, total C, total N and exchangeable Mg. Mehlich III Ca positively correlated with exchangeable Ca and exchangeable Mg at 0-7 cm, and % BS and exchangeable Ca at 7-15 cm. Total carbon positively correlated with total N, exchangeable K and sulfatase acitivity at 0-7 cm, and total N and phosphatase activity at 7-15 cm. Total nitrogen correlated positively with soluble salts, % BS and exchangeable Mg at 0-7 cm and soluble salts and phosphatase activity at 7-15 cm. At both depths % BS correlated positively with exchangeable Ca and Mg. At 0-7 cm, exchangeable Ca correlated positively with exchangeable Mg and for both depths correlated negatively with phosphatase activity. At both depths sulfatase activity correlated positively with galatosidase activity.

PCA was used to determine if PC scores grouped treatments together according to differences in the measured soil properties, and corresponded to the results from the multi-way ANOVA. The small sample size of this data limits the ability to make confident statements using PCA; however, the analysis did produce some notable results. Eigenvectors for soil chemical properties and enzyme activity are an indication of how much influence a particular property has on the variability represented in each principle component; the closer the eigenvector is to (+/-) 1, the more that particular property contributes to the variability. The eigenvectors in these analyses were low, indicating a poor relationship between the soil properties and the variability found in the principle components, however, for the purpose of interpreting the observed grouping of treatments, values greater than or equal to (+/-) 0.3 were considered.

No obvious groupings were found for the first three years (Figures 2.62-2.73), but in the final year, the conventional treatment appears distinguished from the other two treatments (Figures 2.74-2.77) (Tables 2.6-2.9). For the spring of 2007, at 0-7 cm, differentiation of the conventional plots can be seen along the first, second

and third principal components (PC1, PC2 and PC3), and the organic treatment is grouped in the second principle component (Figure 2.74). Eigenvectors above (+/-) 0.3 on these PC were: Mehlich III calcium, % total carbon, soluble salts and exchangeable calcium on PC1; Mehlich III potassium, exchangable potassium, sulfatase activity and galactosidase activity on PC2; and exchangeable potassium exchangeable sodium, % water holding capacity and L-arginine deaminase activity on PC3 (Table 2.6). The loadings of the variables were all positive with the exception of water holding capacity, where as the PC scores for the conventional treatment are negative; therefore the analysis suggests that the conventional treatments are associated with lower values for these variables. In the case of water holding capacity, the organic treatment is associated with a lower value. For the 7-15 cm depth, the third principal component (PC3) separated the conventional from the organic and low-input treatments (Figure 2.75). Mehlich potassium, exchangeable potassium, and exchangeable sodium had the highest correlations on PC3, and all were positive. Again, the conventional scores were negative, indicating lower values for each of those variables (Table 2.7).

The fall of 2007 PCA for both depths show a tendency for the conventional treatments to separate on the first principal component (PC1), with negative scores compared to mostly positive scores in organic and low-input treatments (Figure 2.76, 2.77). The variables most highly correlated to PC1 are pH, Mehlich magnesium, Mehlich calcium, % total carbon, total nitrogen, soluble salts, cation exchange capacity, % base saturation, exchangable calcium, exchangeable sodium, water holding capacity, sulfatase activity, and L-argenine deaminase activity, all correlations positive (Table 2.8, 2.9).

T-RFLP Analysis of Microbial Community

A total of 88 T-RFs were detected across all bacterial profiles, and 12 T-RFs were detected across all fungal profiles. The rhizobacterial profiles contained only one T-RF at 155 bp, which was present in all samples, therefore only bacterial and fungal T-RFLP data was analyzed. Tables 2.10 and 2.11 show alpha, beta and

gamma diversity, based on binary data from bacterial and fungal profiles (Whittaker, 1972; McCune et al., 2002). There were no significant differences between treatment groups in the total means of bacterial and fungal alpha diversity, but there was a significant effect of time and treatment x time in the bacterial diversity. In the conventional treatment, the difference of the total mean number of T-RFs between 2004 and 2007 was 1.2, where as the low-input treatment decreased by 9.4 and the organic by 10.8. There was a significant time effect in fungal diversity, with all treatments decreasing over time. Beta diversity was listed in order to describe the structure of the data. Beta diversity is an important property of the sample when considering multivariate methods of analysis, because high beta diversity causes greater challenges in ordination methods (McCune et al., 2002). In the bacterial profiles, the beta diversity of the whole sampling population over time increased to four. While this is approaching an area of concern, beta diversity greater than five is considered “high” according to a general rule of thumb, therefore ordinations should not be greatly effected (McCune et al., 2002).

Profiles were analyzed for treatment group differences by year as well as differences over time. Relative abundance data and binary data differed with respect to detection of outliers, but overall results were consistent with each other. The results presented here refer to binary, or presence/absence data. While hierarchical clustering and NMS ordination did agree for the most part, bacterial and fungal T-RFLP profiles for years 2004-2006 showed no clear pattern with respect to treatment groups, nor was any pattern relating to blocking or field position observed. The results of MRBP (MRPP with a blocking factor) analysis for both fungal and bacterial profiles were not significant, with A values less than or equal to 0.03, indicating high heterogeneity within groups. MRBP for the year 2007 was also non-significant, but clustering for bacterial profiles formed groups that seemed to separate the conventional treatment (Figures 2.78). A useful NMS ordination for 2007 could not be generated (stress greater than 30). When profiles were compared over time, clustering could be seen according to year, especially the bacterial profiles (Figures 2.79, 2.80). NMS ordination of the

bacterial profiles shows correlations with soil properties (Figure 2.81). Total carbon, total nitrogen, mehlich Mg, Mehlich K, CEC and exchangable K all had correlations greater than 0.3 (soil properties were from the 0-7 cm depth, as were the soil samples from which the T-RFLP profiles were generated). MRPP results were highly significant for between-year differences (Bacterial: T = -14.57, A = 0.42, p < 1.00E-07; Fungal: T = -7.23 A = 0.18 p = 1.05E-06), with significant results for all pair-wise comparisons (Bacterial: A > 0.22, p < 0.05; Fungal: A > .09. p < 0.05) except between years 2004 and 2005 in the fungal profiles.

Economic assessment

The cost of all inputs on a per hectare basis was calculated, including seed costs for both vegetable crops and cover crops (Table 2.12). Organic costs were $ 585.54 greater per hectare than the conventional treatment, and $ 976.41 greater than the low-input. The conventional treatment was $ 890.87 greater than the low-input. The price of agricultural inputs can fluctuate depending on manufacturing cost – the prices used here are from 2008. Labor hours for weed, pest and disease control were approximately 50% more in the organic system than both conventional and low-input systems. The added labor hours were due to cultivation with both machine and wheel hoe, sweet corn treatment with the Zea-lator, and having to use a string-trimmer to finish killing the potato vines before harvest when mowing was insufficient.

Discussion

The aim of this study was to determine the effect of conventional agro-chemicals on various soil chemical and biological properties as compared to organic inputs using a systems approach, and to evaluate three management systems with respect to yield and weed, pest and disease control. Earlier research has shown differences in soil properties between treatments, but with the inclusion of confounding factors such as tillage and cover crop differences, the specific effect of the inputs used in conventional and organic farming can not be determined.

This experiment was designed to test the hypothesis that the use of conventional agro-chemicals negatively affects soil chemical and biological properties in the soil.

In the evaluation of yields and quality, the three systems performed similarly, with no statistically significant differences in total marketable yields. Both the BeSweet 292 edamame and the Dark Red Norland potatoes produced comparable to expected yields (Jones et al., 2003; University of Kentucky Cooperative Extension Service, 2005). The Lancelot sweet corn variety was below expected yields for all treatments (Earnst and Woods, 2005). Extensive lodging occurred, allowing easy access to raccoons and other pests. This was observed to be a major factor in yield reduction in all treatments. There are many reasons for corn lodging, or weakening of stalk causing the plant fall over or lean close to the ground – stress due to disease, certain weather conditions, and over fertilization can contribute. Another study at the UK Horticulture Research Farm using the Lancelot variety also reported problems with lodging, therefore it is possible that this may be a susceptible variety. In the quality assessment of ten randomly selected, shucked ears, the organic treatment had significantly less width and length. A possible reason for the difference in size could be due to the relatively slow release of nitrogen from the organic fertilizer. The low-input treatment received the same amount of N/ha as the organic, but in the readily available form of ammonium nitrate. Evidence of this can not be determined from the data, unfortunately. Mineral nitrogen as ammonia was not different between treatments; however, nitrate was not measured. Rapid nitrification is expected to occur in well aerated soils with a pH of 6-8, therefore nitrate is likely the dominate form of mineral nitrogen taken up by the plants (Foth and Ellis, 1997). Yield and quality of the low input treatment was not significantly less than conventional, which received an additional 33.6 kg N/ha and a full rate of pesticide. Insect damage was not significantly different between treatments, but was undesirably high, with an average of 6 out of 10 ears showing caterpillar damage. While some replications produced total projected yields near or above the national average of 12,096 kg/ha, factoring in at least 60% insect damage

reduces that yield substantially, assuming a market that rejects all insect damage (Earnst et al., 2005). In farmers markets or community-supported agriculture (CSA) programs, organic sweet corn may have a higher threshold for insect damage (caterpillar damage at the tip of the ear can usually be cut out with out much loss to the main edible portion), because the consumers are more concerned about pesticide use. Data was not taken on specific insects; however caterpillars, specifically the corn ear worm, were observed as the greatest culprit in insect damage. Poor caterpillar control in the sweet corn is likely the result of late planting, which caused the corn to put out silks when corn earworm and European corn borer pressure is highest. The timing of control methods may have been off, especially in the organic treatment - the Zea-later must be used to apply the Bt/oil mixture after silks appear but before Corn earworm eggs have hatched and moved into the ear. To avoid problems with pollination, it is recommended to wait approximately 1 week after 50% of corn ears are showing silk; inexperience with this technique may have led to an inaccurate estimation of this percentage; at a time of high insect pressure the window of opportunity for the most effective control may be very short.

The higher cost of applied inputs in the organic treatment compared to conventional is largely due to the cost of fertilizer. While it is generally accepted that organic systems have higher costs, usually associated with labor, fertility is one area where careful management of the soil is intended to reduce the need for purchased inputs. Land being transitioned into organic may not have the full benefits of long-term soil fertility management, which is thought to be a reason why some yields are lower in newly converted systems (Pimentel et al., 2005). In this experiment, kg of N/ha was balanced across all systems to avoid differences in yield and soil properties due to fertility differences. In real farming systems, it is less likely that an organic grower would match nitrogen inputs with conventional recommendations pound for pound using purchased fertilizer. The low-input system was clearly the most cost efficient of the three systems, producing similar yields for about $900 – 1000 less than conventional and organic. In labor hours, the Zea-lator application along with string-trimming the

potato vines added considerable hours to the organic system. Regarding the Zea-lator, perhaps more experience with the technique would have lead to better efficiency. The owner's manual states that one person can treat 0.10 ha in 2 - 2 1/2 hours, however it took five hours for two people to treat the same area. In general, these results support the notion that organic systems have higher labor requirements, and perhaps in some cases higher input costs, although that could very well differ considerably depending on fertility management and the crops grown. Price premiums for organic vegetables can also range up to 400%, depending on the market and the crop (Rodale Institute, 2009). For example, in Kentucky, premiums from around 25-38% have consistently been supported for organic sweet corn, regardless of fluctuating prices for conventional sweet corn (University of Kentucky Cooperative Extension Service, 2009).

According to AGR-1 Lime and Nutrient Recommendations published by the University of Kentucky Cooperative Extension Service, the levels of phosphorus, potassium and magnesium remained well into the "high" category (no additional fertilizer for these nutrients recommended) through out the study. For all treatments the concentration of soluble salts was well below levels of concern for salinity, and pH stayed within slightly acid to near neutral. There were, however, some significant treatment differences in soil chemical properties, and several properties that changed significantly over time in all treatments of the study.

The properties that exhibited significant treatment effects according to total means, but did not change over time, Mehlich III extracted P (7-15 cm) and pH (0-7 cm), did not appear to be a result of the treatments, but instead were differences that remained consistent from the beginning of the study, before any treatments had been applied. Mehlich III extracted P (7-15 cm) deviated from this pattern in the fall of 2007, in which the organic and low-input treatments suddenly increase to a level similar to the conventional. The cause of this increase is unclear – while the Nature Safe fertilizer used in the organic treatment provided some additional phosphorus, the low-input treatment received the same fertilizer treatment as the conventional. One possibility is that soil inversion occurred as a

result of the potato digger. A decrease in pH for the fall of 2007 was observed at the 0-7 cm depth; as pH decreases, the solubility of apatite, a main source of phosphorus in soils, is increased (Foth et al., 1997). Multiple regression analysis also showed an increase in phosphorus to be correlated with a decrease in pH. Increased phosphorus levels at the 0-7 cm would likely have been mixed with the lower soil profiles by the action of the potato digger. In fact, phosphorus at the 0-7 cm depth was shown to increase significantly over time, reaching its highest levels in the fall of 2007. There is also a significant treatment effect for phosphorus at 0-7 cm, with the conventional plots starting out with approximately 100 kg P/ha more than the low-input and organic plots in the spring of 2004. While the conventional treatment increases only slightly overtime, the low-input and organic treatments increase steadily, reaching levels similar to the conventional treatment in the fall of 2007. While there is no corresponding change in pH at the 0-7 cm depth, another possibility is the increase in % total carbon, a result of increasing organic matter which can also be a significant source of phosphorus in soil (Foth et al., 1997). While the conventional plots also show an increase in % total carbon, organic and conventional show the most increase over time.

Soil properties that showed significant treatment differences as well as changes over time included Mehlich III extracted phosphorus (0-7 cm), calcium (0-7 cm), and magnesium (0-7 and 7-15 cm), CEC (0-7 cm) and exchangeable magnesium (0-7 and 7-15 cm). Significant treatment differences were considered artifacts of soil variability in these cases, because examination of the data revealed that these differences were present at the beginning of the study and remained relatively consistent through out, even as the soil properties changed over time. All of these properties, with the exception of CEC, increased over time, along with Mehlich III extracted Ca (7-15 cm), soluble salts (0-7 and 7-15 cm), % base saturation (0-7 and 7-15 cm), sulfatase activity (0-7 and 7-15 cm), cellulase activity (0-7 cm), and mineral nitrogen as NH_4 (0-7 and 7-15 cm). CEC at both 0-7 and 7-15 cm depths decreased over time. These are all properties which had significant time effects, but not significant or meaningful treatment effects. The

increase in soluble salts over time is likely an accumulation due to irrigation, however levels are well below cause for concern, as values less than 4 dS m^{-1} are considered normal (Brady et al., 2002). The increase of plant nutrients and enzyme activity overtime is consistent with the corresponding increase in soil organic carbon, as well as the fact that the majority of plant residues from crops were returned to the soil, along with the nutrients taken up by them. With the exception of the potatoes, only a portion of the crops grown were harvested, leaving the rest to be reincorporated back into the soil. The decrease in CEC, however, was not expected, as soil organic matter contributes to the CEC of soils (Foth et al., 1997; Brady et al., 2002). An examination of the data pattern over time shows a steep decline in 2004 to a relatively steady level after the spring of 2005. There are fluctuations marked by an increase each year in the fall, followed by a decrease in the spring, but for the most part the CEC remains between 14 and 17 cmol kg^{-1} throughout the rest of the study. Since it is unlikely that the clay content of soils was altered, the only other soil property to significantly impact CEC is soil organic matter. While no references were found specifically for instances where an increase in soil organic matter was accompanied by a decrease in CEC, a possible explanation is that after a long fallow period, a significant loss of soil humus, the fraction of organic matter that contributes to CEC, occurred as a result of plowing the field in preparation for this experiment. Soil tillage breaks up organic matter and aerates the soil, encouraging the decomposition of organic residues, leading to a rapid decline in organic matter, which fluctuates until it stabilizes at a lower level (Brady et al., 2002). This is consistent with the pattern seen for the CEC, however, the % total carbon significantly increased in all treatments, most notably at the 0-7 cm depth. In this case, the organic material added in the form of cover crops and crop residue may not have contributed a great deal to the CEC because only a small fraction of that residue would become stabilized humus, the rest being lost to the atmosphere as CO_2 due to decomposition, or remaining as larger un-decomposed particles at the soil surface (Brady et al., 2002).

The soil properties with significant treatment x time effects are the most relevant to the questions posed in this study – these are properties in which significant treatment differences emerged over time. These include % total carbon (0-7 and 7-15 cm), total nitrogen (0-7 and 7-15 cm), Mehlich III extracted potassium (0-7 and 7-15 cm), exchangeable potassium (0-7 and 7-15 cm), and exchangeable sodium (0-7 and 7-15 cm). The % total carbon and total nitrogen follow a similar pattern at both depths, and in fact are strongly correlated in the multiple regression analysis. This is to be expected, since the total nitrogen content of a soil is primarily a component of the soil's organic matter content (Foth et al., 1997). At 0-7 cm, all treatments increase overtime, with the organic treatment increasing significantly greater than both the low-input and conventional treatments. The widest margin of difference between treatments occurs in the spring and fall of 2007. At 7-15 cm, levels of both properties are similar for all treatments until the fall of 2007, when the organic treatment increases above the low-input and conventional. These differences can not be attributed to tillage or cover cropping, which were the same in all treatments. The use of organic fertilizers, the addition of compost, and pastured poultry are all sources of organic matter that could have contributed to the significant increase in the organic treatment. It is possibly a cumulative effect, although the Nature Safe fertilizer is by far the most abundantly applied of these inputs. It should be noted that while significant, the differences between treatments were between one and two tenths of a percentage point.

Mehlich III extracted potassium and exchangeable potassium also had significant treatment effects over time, with the largest changes occurring in the year 2007. Because soil potassium levels are a function of the mineral content of soils, primarily micas and feldspars, increases in soil potassium are likely to be a result of additions from fertilizers and plant residues (Foth et al., 1997; Brady et al., 2002). Since much of the potassium taken up by the vegetable crops and cover crops were returned back into the soil, loss associated with plant uptake would be minimal, while release of potassium from soil minerals would continue to increase available potassium. The Nature Safe fertilizer contained 8% potassium by

weight, where as the conventional and low-input treatments did not receive additional fertilizer potassium except in 2007, which was applied after the spring soil sampling. The low-input treatment also increased with the organic treatment in the spring of 2007, and the conventional did not, suggesting that fertilizer differences were not the cause of this increase. If this were the case, the low-input and conventional treatment would be expected to behave similarly. Potassium from the pastured poultry treatment could have increased levels in the organic treatment, but again, this would not explain the increase seen in the low-input treatment (He *et al.*, 2008). Exchangeable potassium levels in the organic treatment remain high in the fall after the increase in the spring, while the low-input levels decrease in the fall. For Mehlich III extracted potassium, however, both the organic and low-input levels decrease in the fall. Essentially, increased potassium levels in the organic treatment can be explained by the use of organic fertilizers containing potassium and the pastured poultry, but the simultaneous increase in the low-input treatment, which did not receive these inputs, indicates factors besides treatment inputs having an influence, or simply variability in the fluctuation of potassium availability. No references were found to support a difference in plant uptake of potassium according to management differences or fertilizer source.

All treatments showed an increase in exchangeable sodium at 0-7 cm in the spring of 2007, followed by a decrease in the fall. The highest levels were seen in the organic treatment, followed by low-input and conventional. Previous years showed a decline in exchangeable sodium from levels at the beginning of the study. The source of this increase is not clear. The previous year being fallow, there was no treatment applied except for pastured poultry in the organic plots, but this does not explain the increases seen in the low-input and conventional. At 7-15 cm, the organic and low-input treatments increase in the spring of 2007, but the conventional continues to decrease. Exchangeable sodium levels can be affected by rainfall, irrigation and other factors such as salt used in de-icing roads (Brady et al., 2002). Differences in soil drainage, affected the leaching of sodium ions the soil profile could explain the differences seen if the increased sodium

levels were a result of road de-icing (the plots are directly down hill from a major road way).

The results of the principle components analysis were interesting as far as no discernable groupings were present before the last year of the study. Interpretation of PCA is ultimately subjective, and this analysis is limited by low sample size. However, the observation of more distinct grouping of the conventional treatment in the final year bears mentioning. Four years may not be long enough to see major changes in the soil properties measured; furthermore, the study was conducted on Maury silt loam soil naturally high in nutrients, with upwards of 3% organic matter, which had not been cultivated for years prior to this study. Combined with the use of cover crops each year for all treatments (much less common in conventional farming than in organic), it is possible that these factors may have reduced treatment effects due to inputs, at least in the short term. The PCA result could be an indication that after four years, soil properties may only just be starting to show differences according to treatment effects. On the other hand, the low eigenvectors for soil properties does not lend confidence to the analysis. Ideally, at least 90% of variability would be represented in the first three or four principle components, and the measured factors of interest influencing this variability the most would be identified by having eigenvectors close to (+/-) 1. The eigenvectors for the soil properties in this study did not exceed (+/-) 0.5, and most values were even lower. This indicates fairly weak influences of the soil properties on the PC scores of the treatments, therefore, definitive statements can not be made concerning the relationship between soil properties and the variability seen in the principle component scores of the treatments. The correlations found in PCA in 2007 do support the results of the ANOVA, however. Total carbon, total nitrogen, soluble salts, Mehlich III extracted potassium, exchangeable potassium and exchangeable sodium separated the organic and conventional treatments in PCA, and all of these properties were found to have significant treatment or treatment x time differences. Principle component analysis was conducted with the limitations of its usefulness for this experimental design in mind, and was

used as more of an exploratory tool for uncovering possible trends that were not apparent with ANOVA.

The results of the T-RFLP analysis are consistent with the soil chemical and enzyme analysis in that there were no discernable treatment effects within each year, but the profiles showed significant change overall from year to year. As with PCA in soil chemical properties, NMS and clustering of T-RFP data produced no discernable patterns, except for the year 2007, in which the conventional treatment appears to become distinguished in clustering of the bacterial profiles. As with PCA, statistical comparisons of the treatment groups did not yield significant results. A statistical comparison of alpha diversity of the bacterial profiles showed a significant difference in treatment overtime, with all treatments decreasing in the number of T-RFs present, but with the conventional treatment decreasing less than the low-input and organic. The implications of this result are not clear, because the conventional treatment started out lower in alpha diversity than the organic and low-input (20.7, versus 26.7 and 29.7, respectively). After 2004, alpha diversity in all treatments remained relatively similar, in the range of 17-20. This seems to imply that differences present in the beginning of the study became more homogenized over time. Clear differences were apparent in clustering and ordination when comparing T-RF profiles over time, and statistical comparisons (MRPP) confirmed a significant effect. The implication of this result is not apparent – there has been little research into the natural fluctuations of soil microbial communities, much less relating to different soil types and land uses. A recent study has proposed that similarity of less than 70% in T-RFLP profiles could indicate a shift in bacterial populations toward an inherently different state (Meier et al., 2008). There was a 30% difference in the make up of T-RFs present in 2004 and 2007. Additionally, alpha diversity, the total number to T-RFs in a sample, decreased by 32% from 2004 to 2007. Another study found that cultivation has a strong, lasting effect on soil microbial communities, which remained even in fields which had been abandoned for years (Buckley and Schmidt, 2001). Cultivation could be a probable explanation for the changes in microbial T-RFLP profiles over time, however comparison with un-

cultivated soil would be needed to confirm this. The soils samples from 2004 were taken before any planting or treatment had taken place, but plots had already been cultivated several months ahead of time, so there were no pre-cultivation base-line soil samples to evaluate the initial diversity and heterogeneity of the plots. Different cover crops used from year to year are another likely contribution to this result, beginning with hairy vetch/rye in the fall of 2004, to rye grass/clover in the fall 2005 and oats/soybean early spring of 2006, fallow with no cultivation in 2006, and the incorporation of the 2005 cover crop in the spring of 2007. Few reports describing the effects of different cover crops on soil microbial communities could be found in the literature. One study did show that the tropical legumes velvet bean (*Mucuna pruriens*) and sunn hemp (*Crotalaria juncea*) resulted in higher microbial biomass N than other cover crops, which is an indication that the the microbial community can be affected differently depending on the cover crop (Wang et al., 2007). Correlations with soil properties supported what was found in the ANOVA, with total carbon, total nitrogen, Mehlich III extracted potassium and magnesium, exchangeable potassium correlating in the direction of the 2007 T-RF proflile. All of these properties were found to increase over time. Cation exchange capacity correlated in the direction of 2004, a property that was found to decrease over time. Changes in soil chemical properties, notably the addition of organic carbon and nitrogen, would be expected to have an effect on soil microbial populations, specifically those involved in decomposition (Sylvia, 1998).

The effects of pastured poultry on the organic plots in 2006 are difficult to determine from the data. There were no significant differences in soil chemical properties or enzyme activity in the fall of 2006, but in the spring of 2007, exchangeable sodium and exchangeable and Mehlich III extracted potassium are significantly higher in the organic treatment (0-7 cm depth). Poultry manure is high in potassium and could be responsible for this increase, but it is unlikely to have contributed to the exchangeable sodium levels (Liebhardt and Shortall, 1974; He et al., 2008). It is unclear why no effects were seen in the fall of 2006, but it can be reasoned that the manure may have been poorly incorporated into

the soil at that point, and was not represented in the soil samples. Since the soil remained untilled and under cover crop until the potatoes were planted, it is possible that the manure could have remained attached to plant debris and only slowly incorporated into the top layer of the soil through rain and decomposition, with the effects showing up the following spring. It was expected that soil microbial properties would be affected by this treatment, but that was not found to be the case with the parameters measured in this study. Soil enzyme activity was not significantly different in the fall of 2006 or in 2007, and NMS ordination of T-RFs for 2007 suggested a grouping of the conventional treatments, with the organic treatments still fairly dispersed. Considering that when poultry manure is used as fertilizer it is typically applied at rates in tons/acre, it is likely that the small amounts deposited with pastured poultry would not have large effects after only one year. There are many benefits to raising pastured poultry on fallow fields, even for only small gains in soil fertility. There is a lack of peer-reviewed research on the subject, however many growers have reported significant improvements in pasture fertility over time, along with a reduction in pest insects and weeds; moreover, net returns from pastured poultry are potentially anywhere from $1-4 per bird (Salatin, 1993; Berton and Mudd, 2002). Pastured poultry can be a valuable asset to any farm system, but may be especially beneficial in organic systems, where management of crucial elements such as fertility and pest and weed control depend on the combined effects of many strategies. Further research is warranted to determine the extent of any long-term benefits of pastured poultry on soil quality.

Conclusions

With respect to yield and quality of the crops grown in this study, the three management systems performed equally well, and with the exception of sweet corn, produced near expected yields. These results indicate that organic vegetables can be grown in Kentucky and achieve yields comparable to conventional systems. The organic treatment showed a significantly higher increase in total carbon and nitrogen than the low-input and conventional

treatments. While all treatments received additional organic matter in the form of cover crops and vegetable crop residue, the organic had additions of compost, organic fertilizers, poultry manure, and greater weed biomass. These differences likely contributed to the slight but significant difference in total carbon and nitrogen. On the whole, all three systems showed an increase in plant nutrients such as phosphorous, magnesium, calcium, and potassium. No treatment differences were observed in soil enzyme activities, or in bacterial and fungal T-RF profiles. Ultimately, this study did not find many of the differences found in other studies comparing organic and conventional systems. There are several reasons for this. Focusing on inputs and keeping other practices such as tillage and cover cropping the same certainly did not maximize differences between the systems. Furthermore, Maury silt-loam soil that was previously fallow and consistently high in organic matter and nutrients may be buffered against any changes caused by treatment effects, at least in the short term. Variability in the field and a relatively small sample size imposed difficulties on the statistical analysis. Any or all of these factors could have contributed to minimizing treatment effects. There is also the question of whether four years is long enough to see differences relating to inputs. The observed separation of samples from conventional plots in ordination methods for both soil chemical properties and T-RFLP profiles may be an indication of subtle changes emerging in the final year of the study, but this observation alone is not enough to make that determination. A recent study of a long-term agricultural experiment established in 1978 in Switzerland did find soil chemical and biological differences in organic and conventional wheat fields with identical crop rotation and tillage regimes, concluding that “Long term organic farming and the application of farm-yard manure promoted soil quality, microbial biomass and fostered natural enemies and ecosystem engineers, suggesting enhanced nutrient cycling and pest resilience” and “organic fertilizers foster biotic interactions within and between below and above ground components therby improving the sustainability of farming systems” (Birkhofer et al., 2008).

This experiment was designed to expand on the findings of other studies which found which found differences in several soil properties when comparing management systems – the types of inputs used in these systems being one of the major differences between them. By eliminating some the larger confounding factors such as tillage, soil, type and use of cover crops, it was thought that some insight could be gained as to whether or not these inputs have a direct effect on soil properties that are thought to relate to soil quality and sustainability. While this issue remains fundamental to understanding agricultural systems and their environmental impact, this question was not sufficiently answered in this study. It was shown that the additional organic matter inputs in the organic treatment increased the total carbon and nitrogen above the other treatments; however, it does not appear that the use of conventional fertilizers and chemicals caused any decrease in soil quality according the parameters measured in this study, at least in the short term. Another possibility concerning the use of agro-chemicals on soil properties is that the incorporation of soil improving techniques such as cover cropping in conventional systems along with reduced inputs ameliorates the negative effects of the chemicals on the soil, while substantially reducing cost. This could be considered a strong case for the low-input system, which may be a more feasible option than organic for wide spread adoption of more sustainable practices (Pimentel et al., 1989).

To improve the overall quality of information gained from this type of study, several suggestions are proposed for future systems research experiements. Although space and resources are often a limiting factor in research, the study of agricultural systems would benefit by including more than one soil type or areas with differing histories of land use. Permanent plots that can be maintained long-term and studied by a variety of disciplines should be considered for systems research when the goal is to study changes in soil quality or ecological communities. While shorter-term studies can be useful for practical and applied types of information, some soil properties may change very slowly, or differ in the way they are affected due to soil type. So little is known about how the soil microbial community functions over time and how various environmental

conditions effect it – long term studies across different soil types and land uses are critical for interpreting community data in a biologically meaningful way.

The study of agricultural systems is paramount to understanding and achieving sustainable food production. As more research in this area is conducted, it is becoming apparent that in order to understand how sustainability is affected by agriculture, we must further investigate the ecosystem of agriculture's most important resource, the soil.

Table 3.1. Cover crops planted in all three management systems, by year. All cover crops were planted in the fall, with the exception of 2006.

Year	Cover crop and rate
2004	Spring: Sudan grass, 40.3 kg/ha Fall: Rye 64.8 kg/ha, hairy vetch 31.4 kg/ha
2005	Fall: Annual rye grass, 33.6 kg/ha Dutch white clover, 56.0 kg/ha
2006	Spring: Mixture of oats and soybean, 76.2 kg/ha
2007	Fall: Rye 64.8 kg/ha, hairy vetch 31.4 kg/ha

Table 3.2. Management systems treatment summary by year

Year/Crop	Organic	Low-input	Conventional
2004 BeSweet 292 Edamame	*Fertilizer:* none *Weed control:* cultivation *Soil amendment:* 5.5 t/ha compost (post harvest)	*Fertilizer:* none *Weed control:* cultivation *Soil amendment:* 2.7 t/ha compost (post harvest)	*Fertilizer*: none *Weed control:* Dual Magnum at 1.5 l/ha and Canopy at 491 ml/ha (tank mixed)
2005 Lancelot Sweet Corn	*Fertilizer*: Nature Safe 10-2-8, 56 kg N/ha side-dress *Weed control*: Wheel hoe, 2 times *Pest control:* Zeo-later (Johnny's Selected Seeds, Winslow, ME)	*Fertilizer*: NH_4NO_3, 56 kg N/ha side-dress *Weed control*: Bicep Magnum II, 3 l/ha *Pest control:* Pounce 3.2 EC, 280 ml/ha, three times	*Fertilizer*: NH_4NO_3, 33.6 kg N/ha pre-plant, 56 kg of N/ha side-dress *Weed control*: Bicep Magnum II, 6.1 l/ha *Pest control:* Pounce 3.2 EC, 561 ml/ha, three times
2006 Fallow/ Pastured Poultry	Fallow/ pastured poultry	Fallow	Fallow
2007 Dark Red Norland Potatoes	*Fertilizer*: Nature Safe 10-2-8, 112 kg N/ha pre-plant *Weed control*: cultivation *Pest control:* Pyganic EC 1.4, 1.2 l/ha, once over entire plots and 2 times targeted treatments *Disease control:* Copper sulfate 1.2 kg/ha	*Fertilizer*: NH_4NO_3, 112 kg N/ha, K_2SO_4, 22.4 kg K/ha *Weed control*: cultivation *Pest control:* Pounce 3.2 EC, 280 ml/ha, foliar spray, 1 time *Disease control:* Quadris opti, 934 cm^3/ha, 2 times *Vine Kill:* Reward, 2.3 l/ha	*Fertilizer*: NH_4NO_3, 112 kg N/ha, K_2SO_4, 22.4 kg K/ha *Weed control*: cultivation *Pest control:* Admire 2F, at planting, 416 ml/ha *Disease control:* Quadris opti, 1.9 l/ha, 2 times *Vine Kill:* Reward, 2.3 l/ha

Table 3.3. Primers used in multiplex T-RFLP (Singh et al., 2006)

Primer	Fluorescent label	Sequence 5' to 3'	Target region	Specificity
63f	none	AGGCCTAACACATGCAAGTC	16S rRNA gene	Eubacteria (Marchesi et al., 1998)
1494r	VIC (green)	TACGGYTACCTTGTTACGAC	16S rRNA gene	Eubacteria (Lane, 1991)
1244r	PET (red)	CTCGCTGCCCACTGTCAC	16S rRNA gene	Eubacteria (Andreas Tom-Petersen, 2003)
ITS1f	FAM (blue)	CCTGGTCATTTAGAGGAAGTAA	ITS	All fungi (Gardes and Bruns, 1993)
ITS4r	none	TCCTCCGCTTATTGATATGC	ITS	All fungi (White et al., 1990)

Table 3.4. Correlations of soil chemical and enzyme properties at 0-7 cm.

	Soil Properties	1	2	3	4	5	6	7	8	9	10
1	Mehlich III P	-									
2	Mehlich III K	**0.32	-								
3	pH	***-0.63	-0.04	-							
4	Mehlich III Mg	0.05	***0.61	**0.31	-						
5	Mehlich III Ca	**-0.34	**0.39	***0.73	***0.67	-					
6	Total C	-0.03	***0.70	0.20	***0.59	***0.42	-				
7	Total N	***0.39	***0.61	-0.10	***0.67	0.21	***0.75	-			
8	Soluble Salts	**0.30	***0.60	-0.11	***0.64	***0.39	***0.49	***0.61	-		
9	CEC	-0.45	-0.26	0.21	-0.16	0.10	-0.19	*-0.24	-0.17	-	
10	% BS	-0.07	***0.42	***0.60	***0.69	***0.77	***0.42	**0.33	**0.30	**-0.32	-
11	Ex. K	**0.32	***0.92	-0.02	***0.59	***0.38	***0.65	***0.60	***0.62	-0.16	***0.45
12	Ex. Ca	***-0.46	0.09	***0.80	***0.44	***0.85	0.15	-0.02	0.04	**0.36	**0.79
13	Ex. Mg	-0.08	**0.36	***0.46	***0.86	***0.59	***0.48	***0.58	***0.45	-0.04	***0.66
14	Ex. Na	0.01	*0.27	0.16	0.11	0.20	0.14	0.06	0.06	0.13	-0.01
15	L-arg. deaminase	-0.02	0.03	0.16	*0.22	0.18	0.09	*0.22	**0.35	*0.27	0.05
16	Phosphatase	***0.41	0.05	***-0.44	0.07	***-0.36	0.18	***0.44	0.38	-0.21	-0.26
17	Sulfatase	-0.06	*0.25	0.19	**0.30	0.19	***0.57	***0.45	**0.17	**-0.35	**0.34
18	Galactosidase	-0.14	-0.04	0.16	0.03	0.08	**0.30	0.21	0.07	-0.01	0.06
19	Cellulase	0.01	0.22	0.01	0.26	0.14	***0.47	***0.45	0.26	0.08	0.07
	Mean	252.08	740.91	6.10	256.01	3292.05	1.93	4090.17	0.11	15.80	58.15
	Std Dev	90.53	249.07	0.39	63.66	759.92	0.17	429.57	0.06	1.59	11.43

* $p > 0.05$ ** $p > 0.01$ *** $p > 0.001$ Significant correlations greater than 0.5 are shown in bold

Table 4 continued

	Soil Properties	11	12	13	14	15	16	17	18	19
11	Ex. K	-								
12	Ex. Ca	0.14	-							
13	Ex. Mg	**0.35	***0.51	-						
14	Ex. Na	*0.24	0.08	0.05	-					
15	L-arg. deaminase	0.17	0.18	0.19	0.03	-				
16	Phosphatase	0.07	***-0.53	0.13	-0.04	0.20	-			
17	Sulfatase	0.19	0.05	**0.34	-0.21	-0.09	*0.22	-		
18	Galactosidase	0.06	0.02	0.15	-0.19	0.09	0.31	***0.54	-	
19	Cellulase	0.18	0.04	0.28	-0.23	0.17	***0.39	**0.36	***0.38	-
	Mean	0.87	7.26	0.96	0.06	2.33	2.86	0.46	0.36	0.43
	Std Dev	0.30	1.62	0.21	0.02	1.20	0.45	0.34	0.33	0.39

* $p > 0.05$ ** $p > 0.01$ *** $p > 0.001$ Significant correlations greater than 0.50 are shown in bold

Table 3.5. Correlations of soil chemical and enzyme properties at 7-15 cm.

	Soil Properties	1	2	3	4	5	6	7	8	9	10
1	Mehlich III P	-									
2	Mehlich III K	*0.24	-								
3	pH	***-0.58**	-0.15	-							
4	Mehlich III Mg	-0.15	0.10	0.21	-						
5	Mehlich III Ca	***-0.40	0.11	***0.79**	***0.60**	-					
6	Total C	-0.18	***0.41	0.09	***0.46	**0.32	-				
7	Total N	*0.28	*0.24	*-0.26	***0.61**	0.07	***0.64**	-			
8	Soluble Salts	0.16	**0.33	0.04	***0.51**	*0.27	***0.46	***0.51**	-		
9	CEC	-0.20	-0.06	0.08	-0.03	0.06	0.05	-0.10	0.06	-	
10	% BS	***-0.34	-0.08	***0.74**	***0.45	***0.80**	0.16	0.04	0.13	*-0.25	-
11	Ex. K	0.21	***0.89**	-0.09	0.13	0.10	***0.49	**0.34	***0.45	0.00	0.09
12	Ex. Ca	***-0.46	-0.11	***0.81**	**0.35	***0.84**	0.15	-0.09	0.10	**0.35	***0.88**
13	Ex. Mg	*-0.26	-0.18	**0.33	***0.80**	***0.49	**0.35	***0.47	***0.39	0.09	***0.52**
14	Ex. Na	-0.10	***0.44	0.21	0.14	0.19	*0.25	0.00	0.14	0.07	0.07
15	L-arg. deaminase	-0.07	0.05	0.03	*0.25	0.05	**0.34	**0.34	***0.42	**0.35	-0.14
16	Phosphatase	***0.40	-0.04	***-0.54**	0.14	***-0.45	**0.31	***0.57**	*0.26	-0.22	***-0.39
17	Sulfatase	-0.12	*-0.23	0.14	0.16	0.14	*0.26	0.27	-0.01	***-0.48	*0.23
18	Galactosidase	-0.09	-0.22	0.15	-0.09	-0.01	0.17	0.10	0.08	-0.03	0.02
19	Cellulase	-0.11	-0.07	-0.04	**0.33	0.12	***0.55**	**0.34	**0.32	0.03	0.05
	Mean	229.05	529.79	6.09	230.01	3344.67	1.81	3850.96	0.09	15.51	57.01
	St Dev	89.85	129.47	0.44	57.21	786.26	0.14	367.52	0.04	1.49	12.42

* $p > 0.05$ ** $p > 0.01$ *** $p > 0.001$ Significant correlations greater than 0.50 are shown in bold

Table 2.5. *Continued*

	Soil Properties	11	12	13	14	15	16	17	18	19
11	Ex. K	-								
12	Ex. Ca	0.01	-							
13	Ex. Mg	-0.05	***0.48	-						
14	Ex. Na	***0.42	0.09	0.07	-					
15	L-arg. deaminase	0.20	0.01	*0.25	0.16	-				
16	Phosphatase	0.13	**-0.57	0.10	-0.06	*0.26	-			
17	Sulfatase	-0.21	-0.05	0.12	-0.19	-0.20	*0.28	-		
18	Galactosidase	-0.13	-0.01	0.04	-0.05	-0.03	0.20	**0.55	-	
19	Cellulase	0.17	0.03	**0.31	0.12	*0.28	***0.40	0.20	0.11	-
	Mean	0.62	7.38	0.85	0.07	1.76	2.83	0.40	0.23	0.27
	St Dev	0.14	1.75	0.15	0.02	1.22	0.45	0.30	0.28	0.35

* $p > 0.05$ ** $p > 0.01$ *** $p > 0.001$ Significant correlations greater than 0.50 are shown in bold

Table 3.6. Principle components and eigenvalues for Spring 2007, 0-7 cm.

Treatment	PC1	PC2	PC3	PC4
Con-1	**-0.9533**	**-1.1184**	**-0.5543**	-1.1576
Con-2	**-0.2210**	**-1.1125**	**-0.6981**	-0.9648
Con-3	**-0.7277**	**-0.7553**	**-0.2301**	0.5592
Con-4	**-1.2770**	**-0.0329**	**-0.4126**	0.8466
Low-1	-0.2875	0.1695	0.9408	-1.2503
Low-2	2.0074	-1.3023	0.9882	0.9547
Low-3	-0.3714	0.1058	-0.0928	2.0465
Low-4	1.5500	-0.3272	-0.9847	-0.5483
Org-1	-0.1016	**0.3795**	**1.5988**	-0.2892
Org-2	-0.6504	**0.6986**	**0.0649**	0.5188
Org-3	0.1538	**1.5128**	**1.1714**	-0.6101
Org-4	0.8787	**1.7823**	**-1.7915**	-0.1055
Eigenvalue	7.4453	4.0059	3.3858	2.0642
Percent	35.4539	19.0757	16.1230	9.8296
Cum Percent	35.4539	54.5296	70.6526	80.4822
Eigenvectors				
Meh P	-0.09170	-0.12999	-0.13662	0.41217
Mehl K	-0.04520	**0.41029**	0.20839	0.21682
pH	**0.32587**	-0.06775	0.12941	-0.19602
Meh Mg	0.24093	0.23972	-0.15921	-0.07041
Meh Ca	**0.33643**	-0.17372	0.08846	0.02301
%TC	**0.32159**	0.10486	0.10626	0.08863
TN	0.29438	0.08094	-0.15458	0.30388
SS	**0.34057**	0.02022	0.04586	0.09866
CEC	0.24063	0.08151	-0.10112	0.04750
%BS	0.28962	-0.19362	0.15956	-0.01411
Ex K	-0.04265	**0.37315**	**0.24766**	0.20968
Ex Ca	**0.33670**	-0.17401	0.07103	0.00196
Ex Mg	0.22281	0.24185	-0.18209	-0.11622
Ex Na	0.01608	**0.32722**	**0.33444**	-0.16912
%WHC	0.12346	0.01240	**-0.34759**	-0.32079
Phosphatase	-0.14946	0.27463	-0.24822	0.27637
Sulfatase	0.00014	0.29647	0.18572	-0.24635
Galactosidase	0.17917	**0.31604**	-0.29316	-0.03544
Cellulase	0.15962	0.04103	-0.10110	0.45568
L-arg deaminase	0.05062	-0.21974	**0.35213**	0.29903

Grouped principle components and corresponding eigenvectors greater than (+/-) 0.30 are shown in bold.

Table 3.7. Principle components and eigenvalues for Spring 2007, 7-15 cm.

Treatment	PC1	PC2	PC3	PC4
CON1	-0.5988	0.0019	**-0.7633**	-1.4851
CON2	0.0846	0.3184	**-1.0219**	-1.4584
CON3	-0.6615	-0.3805	**-0.8862**	0.3777
CON4	-1.1249	-0.0327	**-1.0327**	1.2949
LOW1	-0.1383	-0.1575	**1.1890**	-1.1102
LOW2	1.9723	-1.4359	**0.4275**	0.1362
LOW3	-0.7679	-0.4877	**0.0889**	1.0962
LOW4	1.8882	1.0261	**-1.4793**	0.4669
ORG1	0.0602	-1.0891	**0.9400**	-0.4699
ORG2	-0.9080	0.2593	**0.3117**	-0.4822
ORG3	-0.0045	-0.4544	**0.7461**	1.2580
ORG4	0.1986	2.4322	**1.4803**	0.3759
Eigenvalue	8.9979	2.9179	2.5738	2.0148
Percent	42.8473	13.8949	12.2561	9.5943
Cum Percent	42.8473	56.7422	68.9983	78.5925
Eigenvectors				
Meh P	-0.08853	0.20576	0.28137	0.44818
Mehl K	0.08069	-0.11779	**0.52471**	-0.20035
pH	0.29381	-0.22508	-0.04436	0.03540
Meh Mg	0.26254	0.22987	-0.06406	0.02749
Meh Ca	0.30819	-0.06697	-0.07945	0.10847
OM%	0.28035	-0.11019	0.19507	0.00716
TN	0.20114	0.19190	0.17181	0.23279
SS	0.13294	-0.33629	-0.15610	0.26334
CEC	0.31836	0.04831	-0.02028	-0.02410
BS	0.29977	0.07018	0.03370	0.17873
Ex K	0.12746	0.02884	**0.51086**	-0.14692
Ex Ca	0.31317	0.06638	0.01206	0.15224
Ex Mg	0.22526	0.27853	-0.06934	-0.00329
Ex Na	-0.02538	-0.18625	**0.38922**	-0.06160
%WHC	0.17414	0.43069	-0.15075	0.14159
Phosphatase	-0.18094	0.36139	0.06862	-0.20276
Sulfatase	0.25034	0.17377	0.14938	-0.24521
Galactosidase	0.22481	-0.27664	-0.17042	-0.30128
Cellulase	0.20148	-0.10744	-0.17211	-0.24762
L-arg deaminase	0.15420	0.03116	-0.05128	-0.32720

Grouped principle components and eigenvectors greater than (+/-) 0.30 are shown in bold.

Table 3.8. Principle components and eigenvalues for Fall 2007, 0-7 cm.

Treatment	PC1	PC2	PC3	PC4
CON1	-1.4658	**-0.7189**	-0.0374	-0.1815
CON2	-0.0822	**-0.5989**	0.6964	-2.4231
CON3	-1.0684	**-0.7907**	0.4843	1.5776
CON4	-1.1146	**1.0134**	-1.5072	-0.5803
LOW1	-0.5148	-1.4997	0.6113	0.2589
LOW2	1.5828	-1.1421	0.2763	-0.2392
LOW3	-0.0562	0.7405	-0.5478	-0.4938
LOW4	1.6917	-0.3186	-1.2327	0.0771
ORG1	0.3261	**1.5518**	2.1200	0.3329
ORG2	-0.3076	**1.0378**	-0.2196	-0.1297
ORG3	0.7447	**0.8730**	0.3810	0.6299
ORG4	0.2643	**-0.1476**	-1.0245	1.1712
Eigenvalue	9.5247	2.9581	2.2748	1.7442
Percent	45.3555	14.0863	10.8324	8.3057
Cum Percent	45.3555	59.4418	70.2741	78.5798
Eigenvectors				
Meh P	0.01144	**0.35170**	-0.20946	-0.35197
Mehl K	0.13154	**0.42519**	0.20276	-0.02617
pH	0.28297	-0.23250	0.14451	-0.06570
Meh Mg	0.28194	0.03263	0.02492	0.07011
Meh Ca	0.28259	-0.20590	0.02107	-0.03751
Meh	0.11691	**0.37718**	0.30897	0.14474
OM%	0.28609	0.01771	-0.15675	0.18984
TN	0.24303	0.19481	-0.31991	0.12141
SS	0.29129	0.14707	-0.14494	0.06211
CEC	0.28458	-0.16192	-0.02397	0.05979
BS	0.25846	-0.09885	0.25177	-0.21942
Ex K	0.09850	**0.41901**	0.34375	0.08853
Ex Ca	0.27685	-0.19493	0.12011	-0.13838
Ex Mg	0.07289	0.05284	0.36321	-0.48291
Ex Na	0.16907	0.10396	0.10727	0.35956
%WHC	0.27347	-0.04864	-0.07471	-0.15819
Phosphatase	-0.18257	0.18222	0.04090	0.24690
Sulfatase	0.24741	-0.01202	-0.25845	0.23220
Galactosidase	-0.09250	-0.15883	0.25088	0.39699
Cellulase	-0.05438	0.24293	-0.40030	-0.22443
L-arg deaminase	0.23416	0.11289	-0.11169	-0.01211

Grouped principle components and eigenvectors greater than (+/-) 0.30 are shown in bold.

Table 3.9. Principle components and eigenvalues for Fall 2007, 7-15 cm.

Treatment	PC1	PC2	PC3	PC4
CON1	**-1.2861**	**-0.8586**	-0.6337	0.0137
CON2	**-0.5167**	**-0.6612**	-0.3602	-0.6908
CON3	**-0.8588**	**-0.7144**	-0.1240	-0.6267
CON4	**-1.3821**	**0.1632**	0.5531	0.3371
LOW1	-0.1814	-0.1826	-0.4298	-1.1965
LOW2	1.8211	-1.2372	-0.0430	-0.8889
LOW3	-0.1068	0.6065	0.2439	-0.1392
LOW4	1.2845	-0.9848	0.9564	1.3005
ORG1	**0.4166**	**0.7877**	1.9262	-0.5915
ORG2	**-0.4748**	**1.1663**	0.8998	1.0769
ORG3	**0.9559**	**2.0916**	-1.4959	-0.6364
ORG4	**0.3285**	**-0.1763**	-1.4930	2.0418
Eigenvalue	8.8727	4.2124	2.0344	1.5592
Percent	42.2510	20.0589	9.6875	7.4246
Cum Percent	42.2510	62.3099	71.9974	79.4220
Eigenvectors				
Meh P	-0.07751	0.09764	0.34865	0.21786
Mehl K	0.01541	**0.44596**	0.07803	-0.18101
pH	**0.30186**	-0.16771	0.03027	-0.12585
Meh Mg	0.24650	0.11479	-0.22927	0.26290
Meh Ca	0.28893	-0.21292	0.05828	-0.07666
OM%	0.29939	0.06787	0.03657	0.00255
TN	0.24474	0.06720	0.16797	0.44395
SS	**0.30502**	0.04782	0.17251	0.05192
CEC	0.25248	-0.11568	0.35303	0.06018
BS	0.29741	-0.07984	-0.00275	-0.29253
Ex K	0.05197	**0.44062**	0.17800	-0.07187
Ex Ca	0.29596	-0.15522	0.05817	-0.21757
Ex Mg	0.11961	0.30167	-0.07082	-0.39467
Ex Na	0.27895	0.15284	-0.05578	0.05517
%WHC	0.02302	0.32209	-0.18471	0.45485
Phosphatase	0.00521	0.28638	-0.33693	-0.02413
Sulfatase	0.21037	-0.20537	-0.20289	0.29647
Galactosidase	-0.12396	-0.01143	0.17130	-0.07563
Cellulase	0.20000	0.22051	-0.26774	-0.16003
L-arg deaminase	0.25985	-0.03824	-0.27200	0.02914

Grouped principle components and eigenvectors greater than (+/-) 0.30 are shown in bold.

Table 3.10. Alpha, Beta and Gamma diversity based on relative abundance of bacterial T-RFLP profiles

	2004			2005			2006			2007		
	α	β	γ	α	β	γ	α	β	γ	α	β	γ
Conventional	20.7	2.2	68	20.0	2.0	60	20.5	1.8	58	19.5	2.1	59
Low-input	29.0	1.3	68	20.2	2.0	60	20.5	1.8	58	18.2	2.2	59
Organic	26.7	1.5	68	19.5	2.1	60	21.2	1.7	58	17.3	2.4	59
α	26.4			19.9			21.1			17.9		
β	2.3			3.4			3.2			4		
γ	88			88			88			88		

α = richness, number of T-RFs present in sample unit
γ = "landscape" diversity – total number of T-RFs in all sample units
β = amount of compositional variation in sample units (γ / α-1)
Values listed are means. Diversity values for the whole sample population are listed at the bottom of the table. Below are the results of the effect tests in a multi-way ANOVA. An asterisk indicates significance of $p \geq 0.05$.

Source	DF	Mean Square	F Ratio	Prob > F
Treatment	2	10.69	0.80	0.4591
Block	3	15.28	1.14	0.3471
Time	1	190.24	14.16	*0.0006
Treatment*Time	2	44.28	3.30	*0.0485
Error	36	13.43		

Table 3.11. Alpha, Beta and Gamma diversity based on relative abundance of fungal T-RFLP profiles

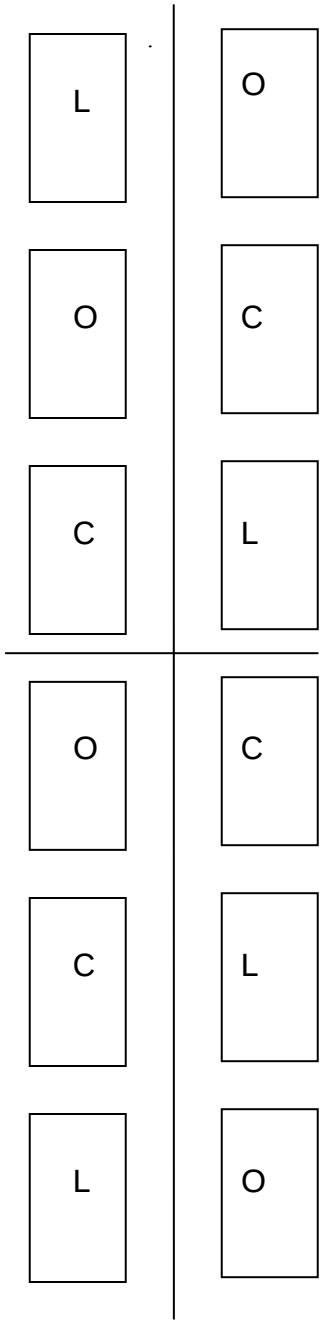
	2004			2005			2006			2007		
	α	β	γ	α	β	γ	α	β	γ	α	β	γ
Conventional	7.7	0.43	11	5	0.60	8	5.5	0.64	9	4.5	0.56	7
Low-input	6.7	0.64	11	4.8	0.67	8	4.0	1.25	9	2.5	1.8	7
Organic	5.3	1.1	11	5.5	0.45	8	4.8	0.88	9	2.5	1.8	7
α		6.4			5.1			4.8			3.2	
β		0.88			1.35			1.5			2.75	
γ		12			12			12			12	

α = richness, number of T-RFs present in sample unit
γ = "landscape" diversity – total number of T-RFs in all sample units
β = amount of compositional variation in sample units (γ / α-1)
Values listed are means. Diversity values for the whole sample population are listed at the bottom of the table. Below are the results of the effect tests in a multi-way ANOVA. An asterisk indicates significance of $p \geq 0.05$.

Source	DF	Means Square	F Ratio	Prob > F
Treatment	2	6.49	2.79	0.0746
Block	3	3.93	1.69	0.1859
Time	1	56.12	24.14	*<.0001
Treatment*Time	2	1.01	0.43	0.6510
Error	36	2.32		

Table 3.12. Comparison of inputs on a cost/ha basis

	Organic	Low-input	Conventional
Compost	44.80	22.40	
Dual Magnum		45.76	
Canopy			101.20
Ammonium Nitrate		366.30	416.07
Nature Safe	1122.00		
Crop oil	193.38		
Bt	34.39		
Bicep Magnum II	18.59	37.18	
Pounce		39.07	58.61
Pyganic	51.99		
Copper Sulfate	65.98		
Quadris Opti	29.70	59.40	
Reward		60.06	60.06
Admire			148.72
Total seed cost	2431.72	2431.72	2431.72
Total cost	3944.25	2967.84	3358.71



Plots measured 12 x 18 meters,

with a 8 m space between plots.

C = conventional

O = organic

L = low-input

Figure 3.1. Plot map of treatments in a randomized block design.

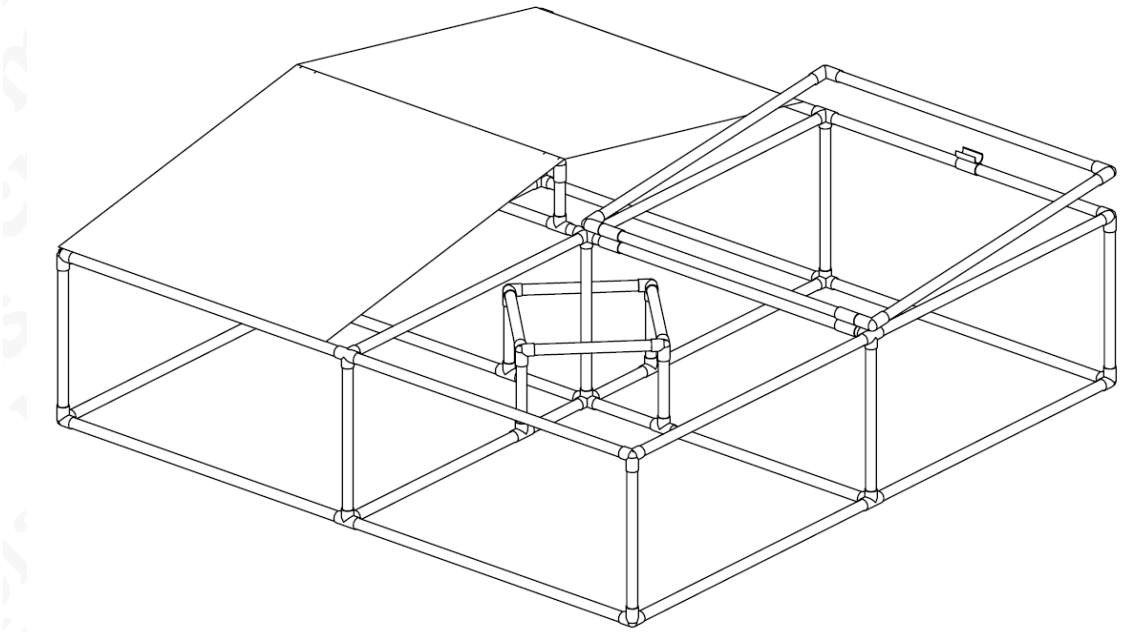


Figure 3.2. Pastured poultry pen constructed using PVC

Constructed pens measured 1.5 x 1.5 m.
Plans prepared by Dean Hunt/JC Designs
http://www.pvcplans.com/pvc-pastured-poultry-pen.htm

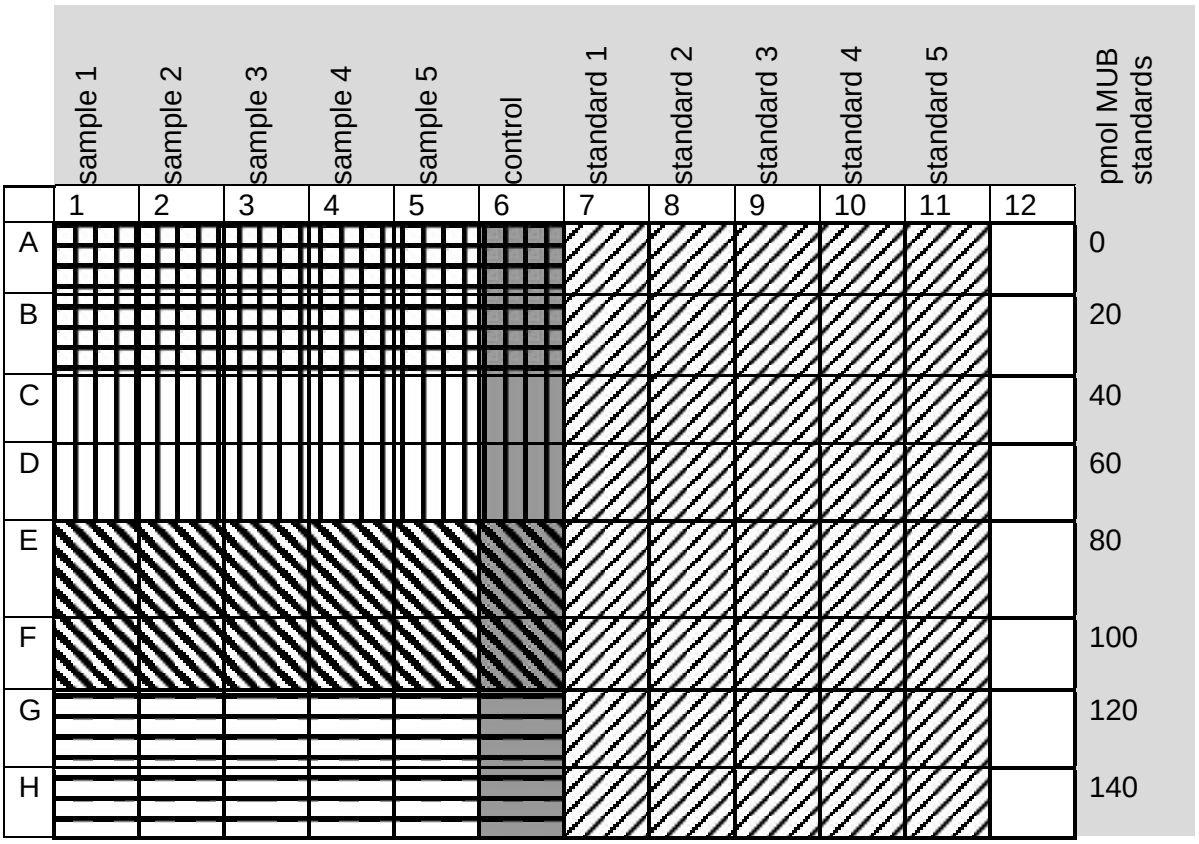


Figure 3.3. Microplate set up for maximum enzyme activity under substrate saturation

Each well had a total volume of 200 µl, with 0.1 M MES buffer and 20µl soil suspension or sterile water (control).

$$\frac{\Delta \text{ Fluorescence/second (sample)}}{\Delta \text{ Fluorescence/pmol MUB (standard curve)}} \times \frac{60 \text{ seconds}}{\text{minute}} \times \frac{\text{nmol}}{1000 \text{ pmol}} \Big/ \text{g dw soil}$$

Figure 3.4. Formula used for the calculation of nmol MUB g dw soil^{-1} minute^{-1}

Grams of dry weight soil in 20µl of soil slurry taken from a 100 ml suspension was estimated using the gravimetric water content of each soil sample.

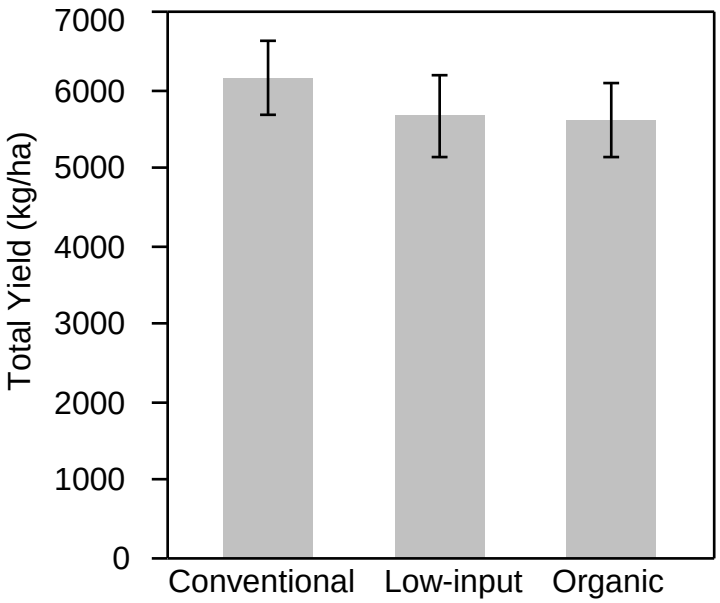


Figure 3.5. Edamame soybean yields for the year 2004.
There was no significant difference between treatments at the P = 0.05 LSD level. Bars indicate the standard error.

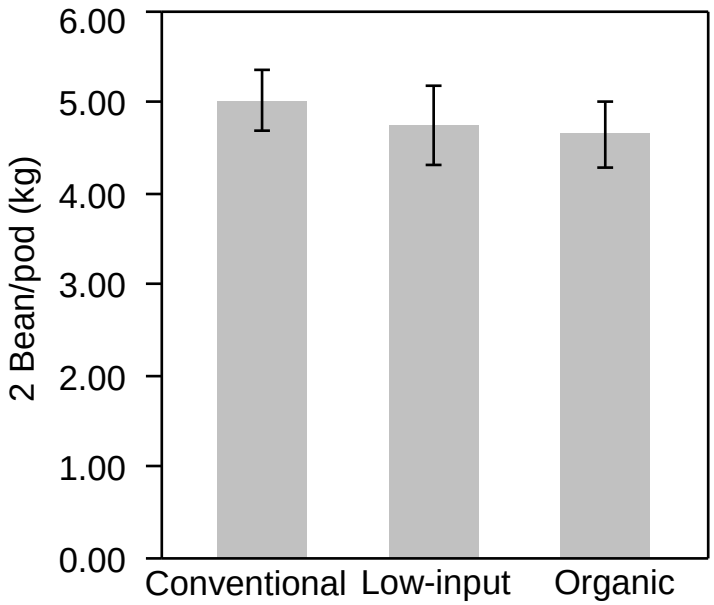


Figure 3.6. Edamame soybean quality category of two beans per pod. There was no significant difference between treatments at the P = 0.05 LSD level. Bars indicate the standard error.

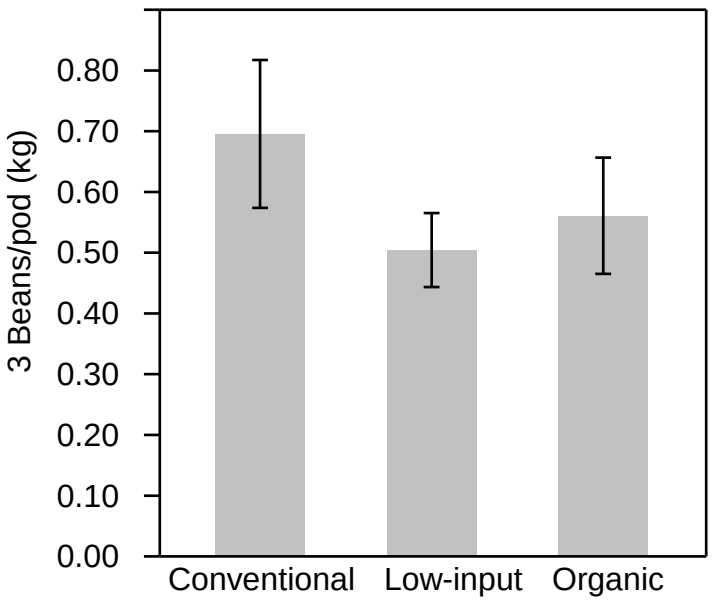


Figure 3.7. Edamame soybean quality category of three beans per pod. There was no significant difference between treatments at the P = 0.05 LSD level. Bars indicate the standard error.

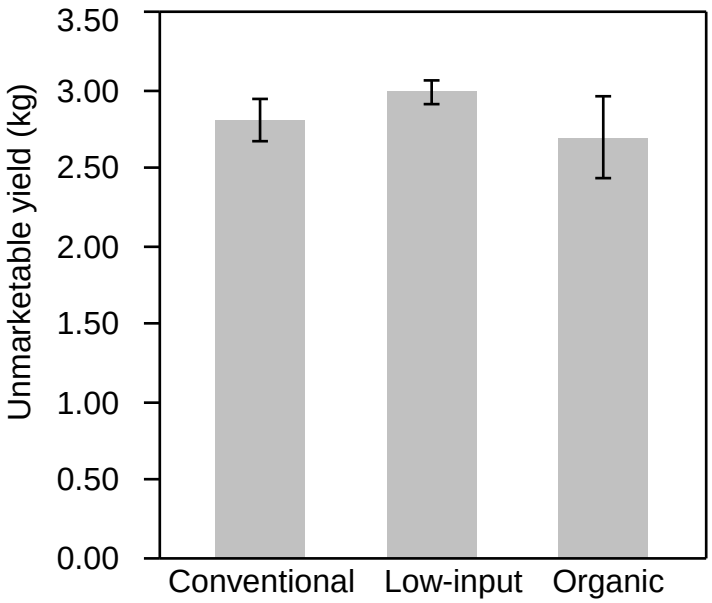


Figure 3.8. Edamame soybean pods designated “unmarketable” (diseased, damaged, only one bean/pod)
There was no significant difference between treatments at the P = 0.05 LSD level. Bars indicate the standard error.

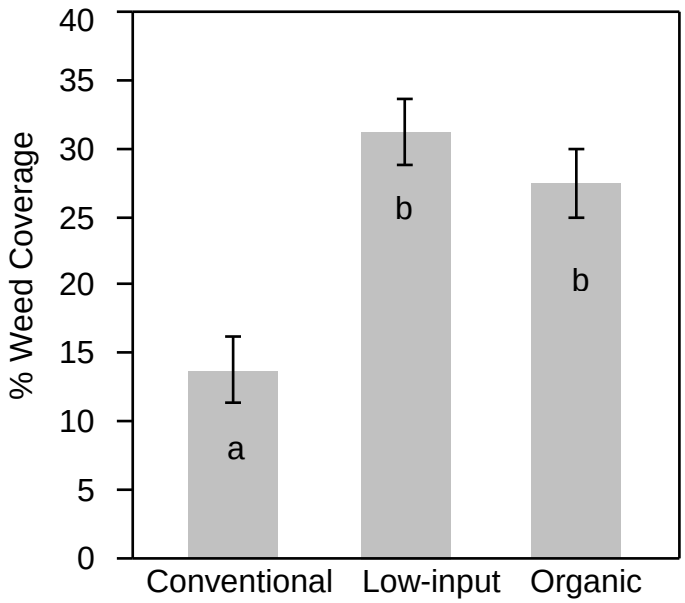


Figure 3.9. % Weed coverage in edamame soybean plots. Different lower case letters denotes a significant difference at the P = 0.05 LSD level. Bars indicate the standard error.

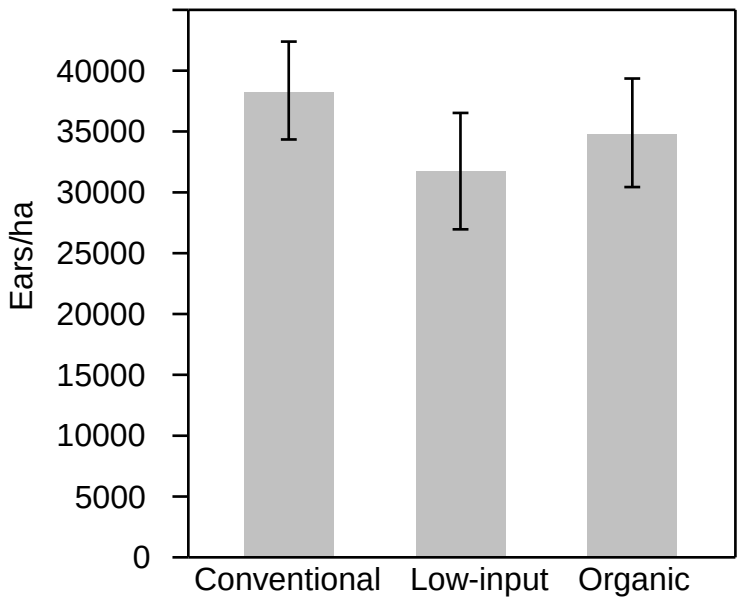


Figure 3.10. Number of ears of sweet corn per hectare.
There was no significant difference between treatments at the P = 0.05 LSD level. Bars indicate the standard error.

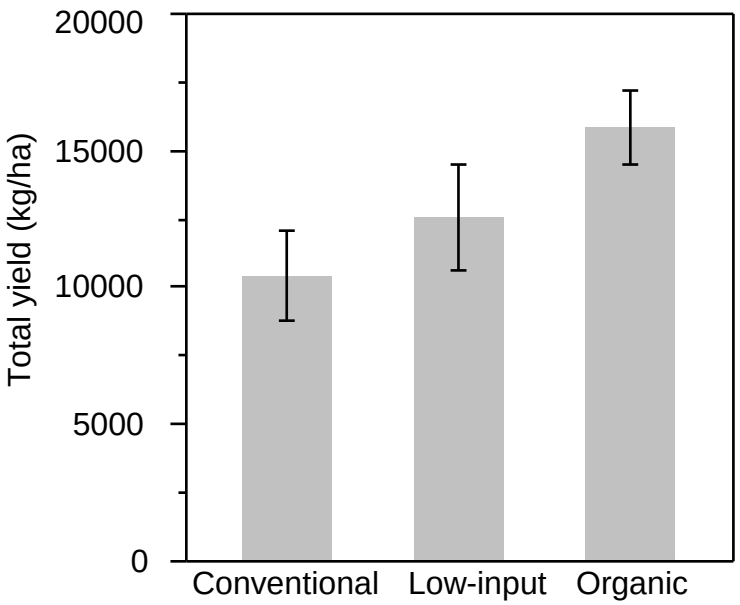


Figure 3.11. Total sweet corn yield.

There was no significant difference between treatments at the P = 0.05 LSD level. Bars indicate the standard error.

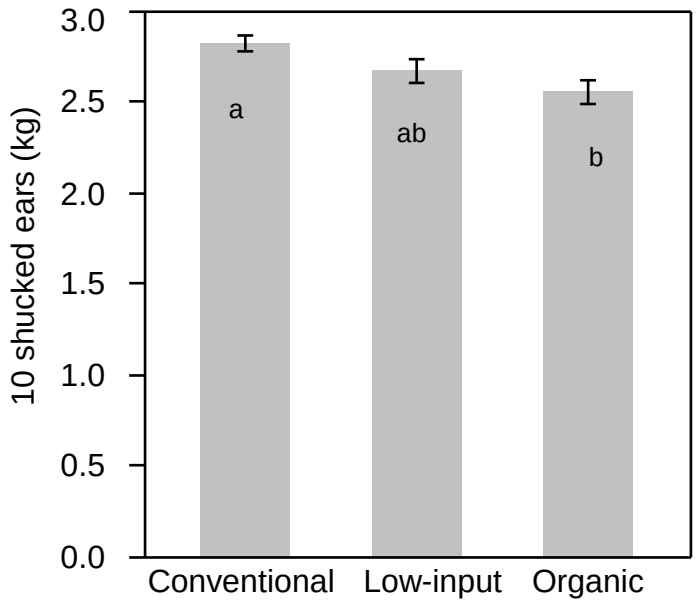


Figure 3.12. Weight of 10 randomly selected, shucked ears of sweet corn. Different lower case letters denotes a significant difference at the P = 0.05 LSD level. Bars indicate the standard error.

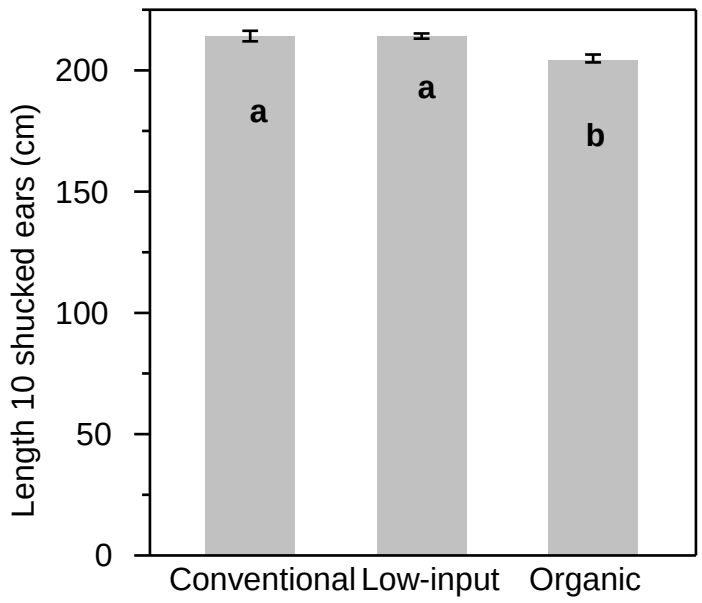


Figure 3.13. Total length of 10 randomly selected, shucked ears. Different lower case letters denotes a significant difference at the P = 0.05 LSD level. Bars indicate the standard error.

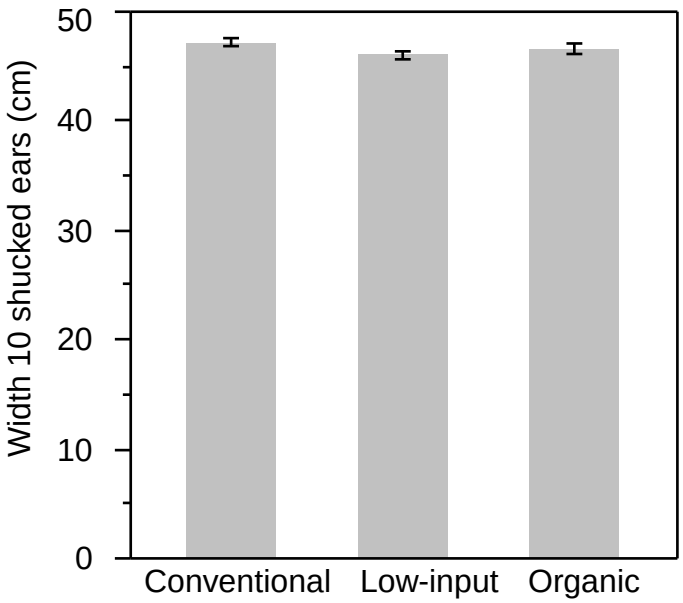


Figure 3.14. Width of 10 randomly selected, shucked ears.
There was no significant difference between treatments at the P = 0.05 LSD level. Bars indicate the standard error.

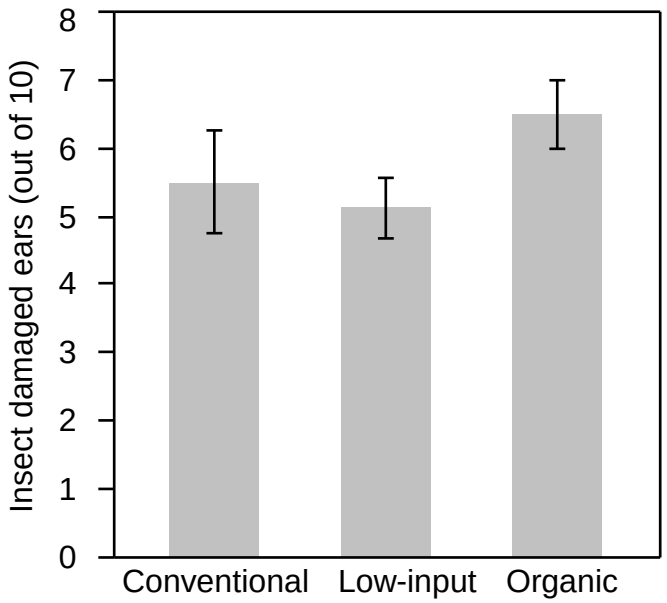


Figure 3.15. Number out of 10 randomly selected, shucked ears that were damaged by insects

There was no significant difference between treatments at the P = 0.05 LSD level. Bars indicate the standard error.

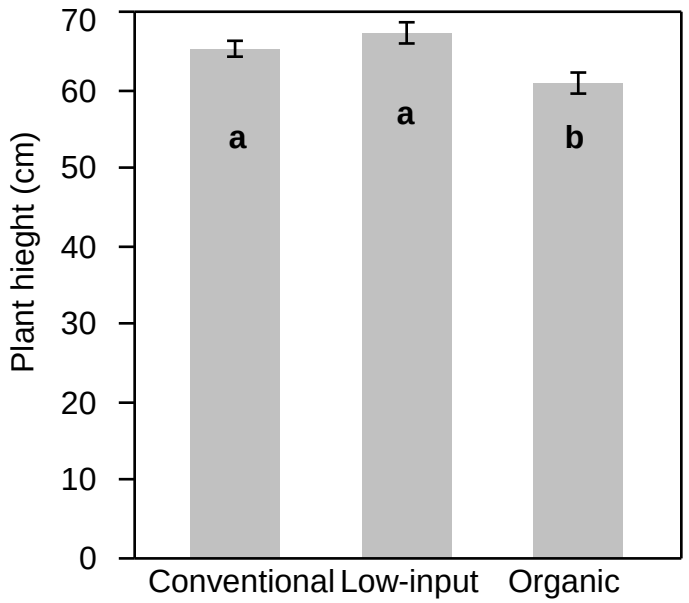


Figure 3.16. Sweet corn plant height, averaged from 10 randomly selected plants from each harvested area

Different lower case letters denotes a significant difference at the P = 0.05 LSD level. Bars indicate the standard error.

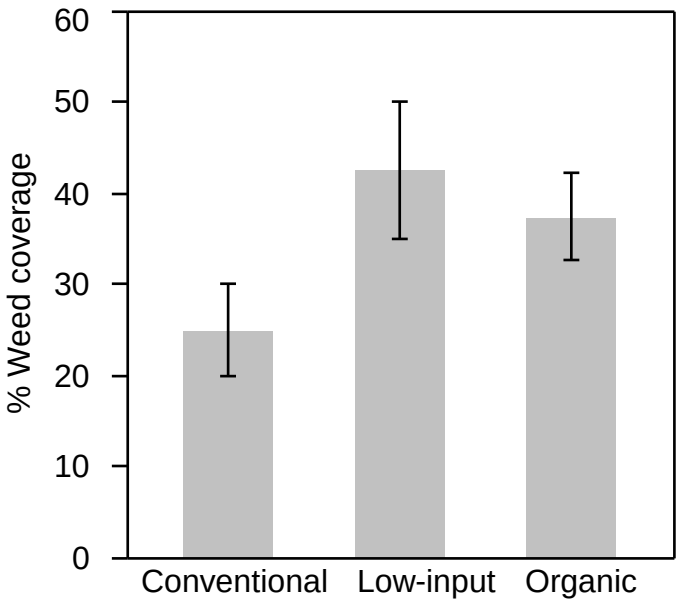


Figure 3.17. % Weed coverage in sweet corn plots.
There was no significant difference between treatments at the P = 0.05 LSD level. Bars indicate the standard error.

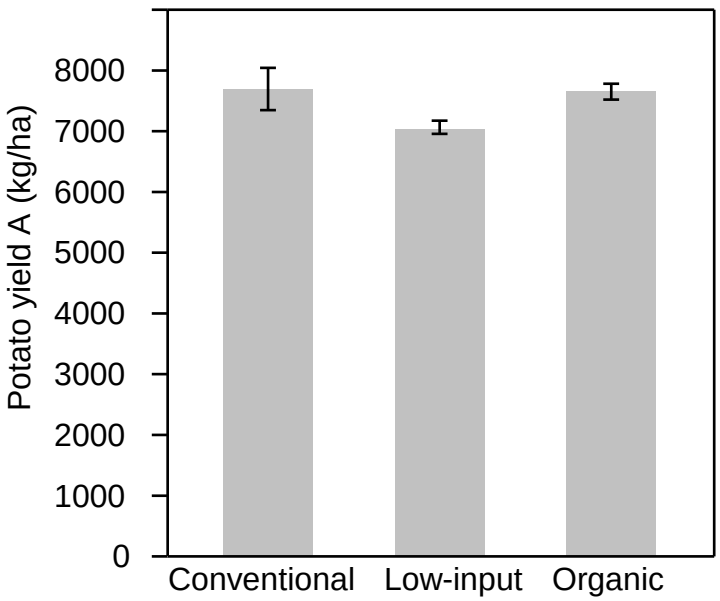


Figure 3.18. Potato yield, size category A (> 5.75 cm circumference) There was no significant difference between treatments at the P = 0.05 LSD level. Bars indicate the standard error.

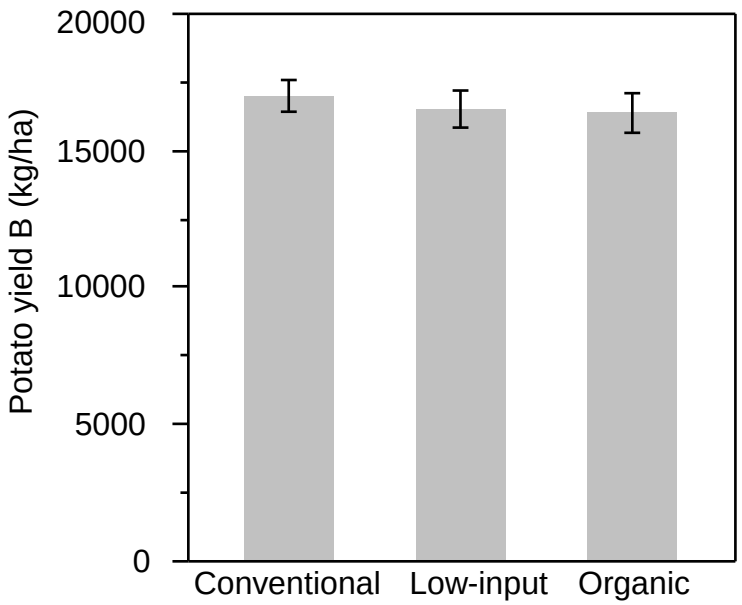


Figure 3.19. Potato yield, size category B (4.5 – 5.75 cm circumference). There was no significant difference between treatments at the P = 0.05 LSD level. Bars indicate the standard error.

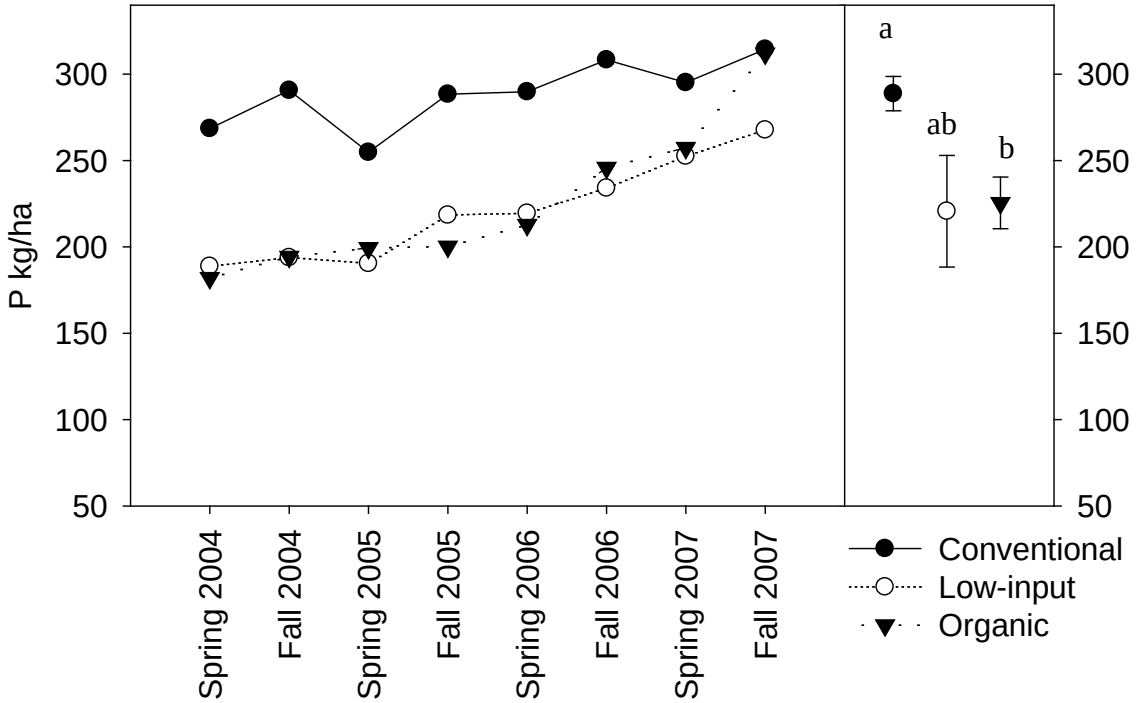


Figure 3.20. Mehlich III extracted phosphorous at 0-7 cm depth. Symbols at right indicate the standard error of the grand mean for all sampling times. The presence of different lower-case letters denotes a significant difference using Tukeys HSD (LSMeans). Below are the results of the effect tests in a multi-way ANOVA. An asterisk indicates significance at the level of α = 0.05.

Source	DF	Mean Square	F Ratio	Prob > F
Treatment	2	32034.87	5.80	*0.0046
Block	3	40691.26	7.37	*0.0002
Time	1	42380.10	7.68	*0.0071
Treatment*time	2	4686.51	0.85	0.4322
Error	71	5521.60		

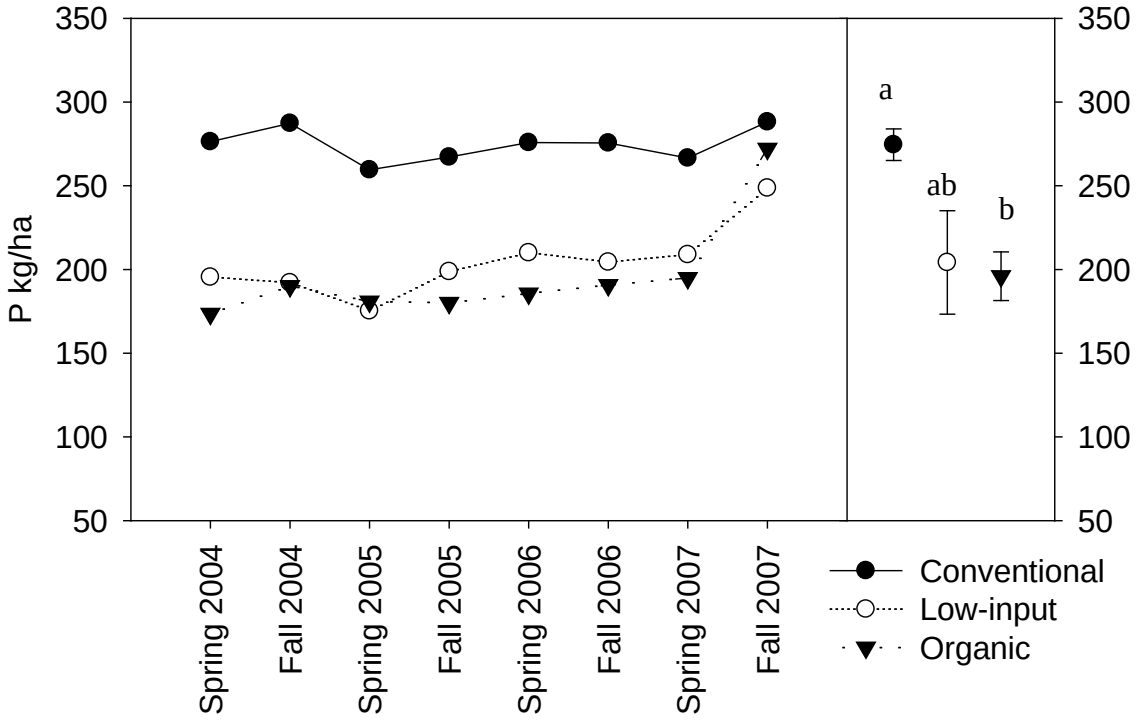


Figure 3.21. Mehlich III extracted Phosphorus at 7-15 cm depth. Symbols at right indicate the standard error of the grand mean for all sampling times. The presence of different lower-case letters denotes a significant difference using Tukeys HSD (LSMeans). Below are the results of the effect tests in a multi-way ANOVA. An asterisk indicates significance at the level of α = 0.05.

Source	DF	Mean Square	F Ratio	Prob > F
Treatment	2	49517.61	9.09	*0.0003
Block	3	42082.02	7.72	*0.0002
Time	1	8220.84	1.51	0.2233
Treatment*time	2	2840.61	0.52	0.5959
Error	71	5447.80		

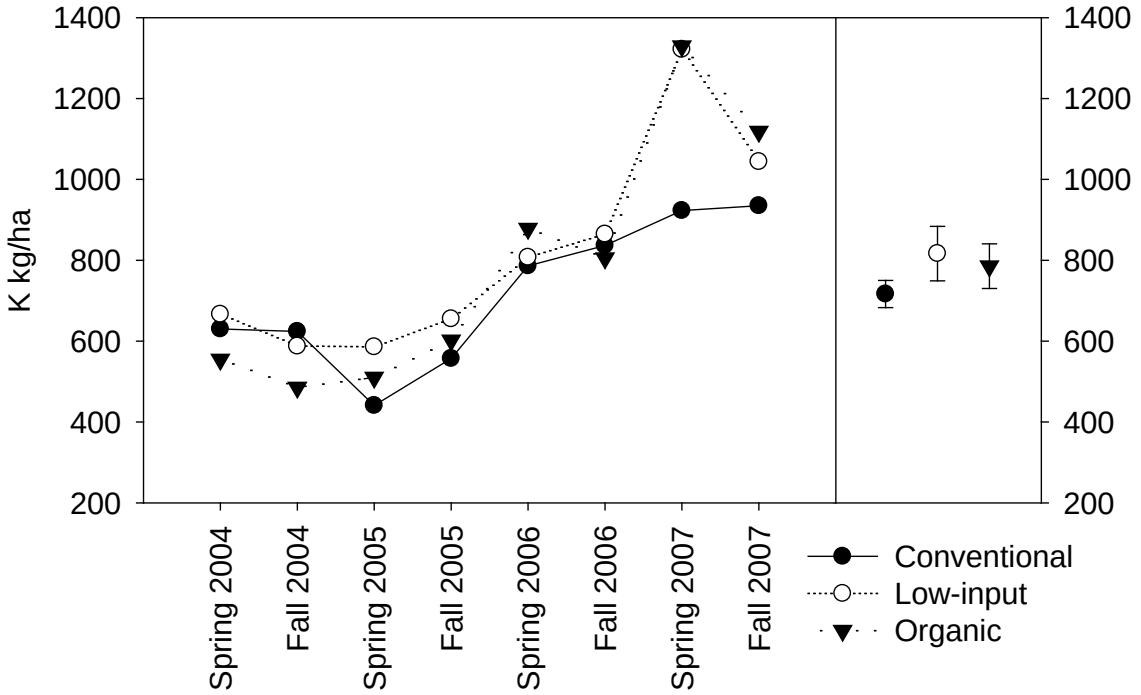


Figure 3.22. Mehlich III extracted Potassium at 0-7 cm depth.
Symbols at right indicate the standard error of the grand mean for all sampling times. The presence of different lower-case letters denotes a significant difference using Tukeys HSD (LSMeans). Below are the results of the effect tests in a multi-way ANOVA. An asterisk indicates significance at the level of α = 0.05.

Source	DF	Mean Square	F Ratio	Prob > F
Treatment	2	43927.85	2.19	0.1195
Block	3	73221.00	3.65	*0.0165
Time	1	3022734.40	150.64	*<.0001
Treatment*time	2	139512.35	6.95	*0.0017
Error	71	20065.00		

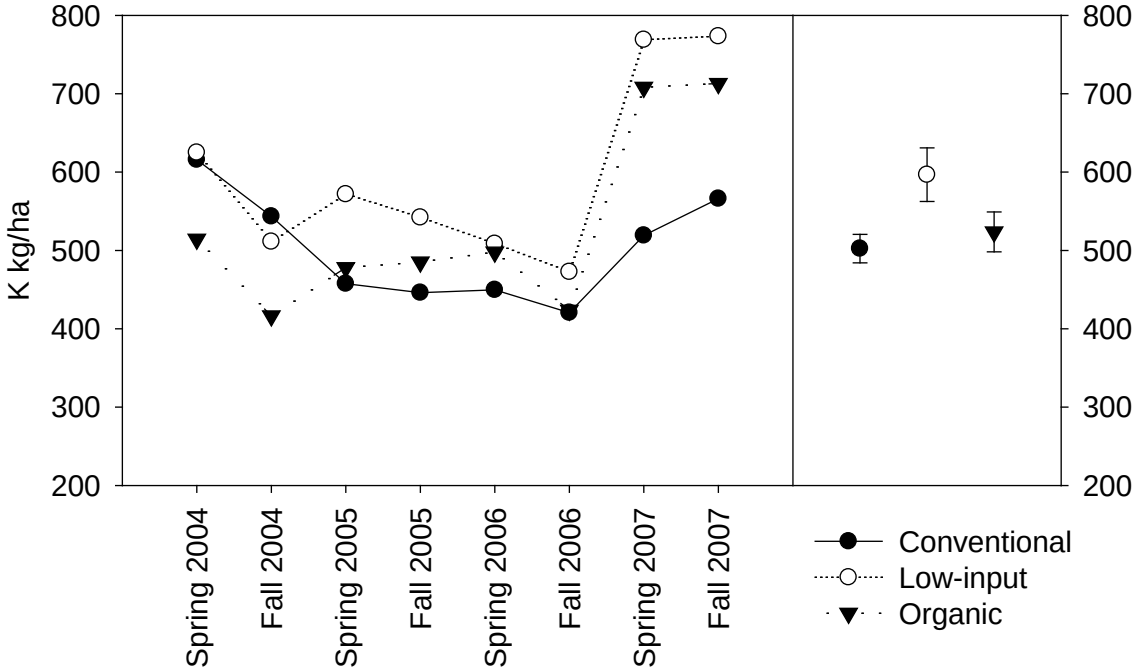


Figure 3.23. Mehlich III extracted Potassium at 7-15 cm.
Symbols at right indicate the standard error of the total mean for all sampling times. The presence of different lower-case letters denotes a significant difference using Tukeys HSD (LSMeans). Below are the results of the effect tests in a multi-way ANOVA. An asterisk indicates significance at the level of α = 0.05.

Source	DF	Mean Square	F Ratio	Prob > F
Treatment	2	35275.54	1.48	0.2357
Block	3	132165.85	3.68	*0.0159
Time	1	125151.80	10.47	*0.0019
Treatment*time	2	147776.73	6.18	*0.0034
Error	70	11955.80		

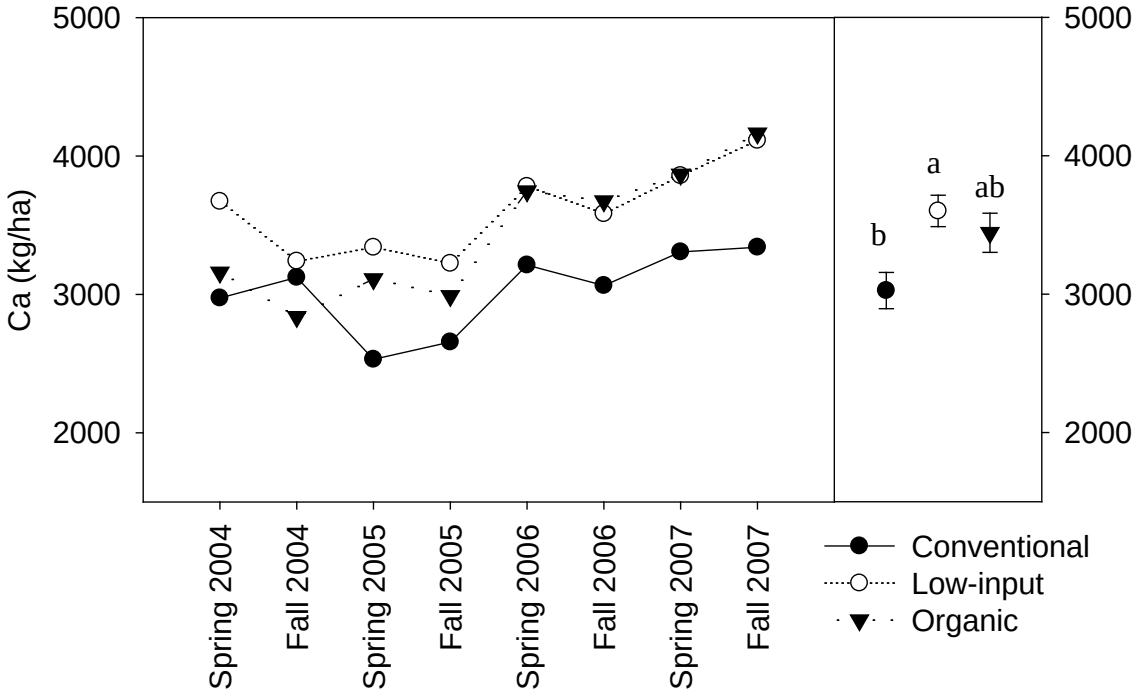


Figure 3.24. Mehlich III extracted Calcium at 0-7 cm.
Symbols at right indicate the standard error of the grand mean for all sampling times. The presence of different lower-case letters denotes a significant difference using Tukeys HSD (LSMeans). Below are the results of the effect tests in a multi-way ANOVA. An asterisk indicates significance at the level of α = 0.05.

Source	DF	Mean Square	F Ratio	Prob > F
Treatment	2	1497313.35	3.88	*0.0251
Block	3	1796773.80	4.66	*0.0050
Time	1	4996708.50	12.96	*0.0006
Treatment*time	2	572577.25	1.49	0.2334
Error	71	385518.00		

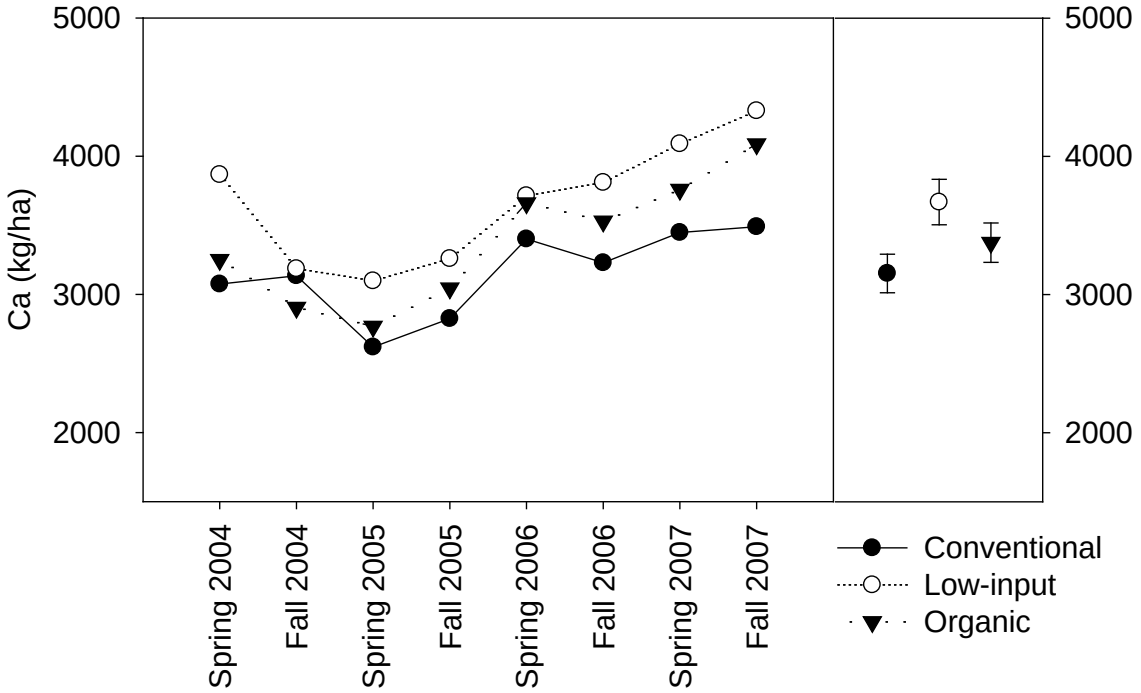


Figure 3.25. Mehlich III extracted Calcium at 7-15 cm.
Symbols at right indicate the standard error of the grand mean for all sampling times. The presence of different lower-case letters denotes a significant difference using Tukeys HSD (LSMeans). Below are the results of the effect tests in a multi-way ANOVA. An asterisk indicates significance at the level of α = 0.05.

Source	DF	Mean Square	F Ratio	Prob > F
Treatment	2	528294.85	1.19	0.3104
Block	3	2575061.53	5.80	*0.0013
Time	1	5688085.90	12.81	*0.0006
Treatment*time	2	239499.45	0.54	0.5856
Error	71	444185.00		

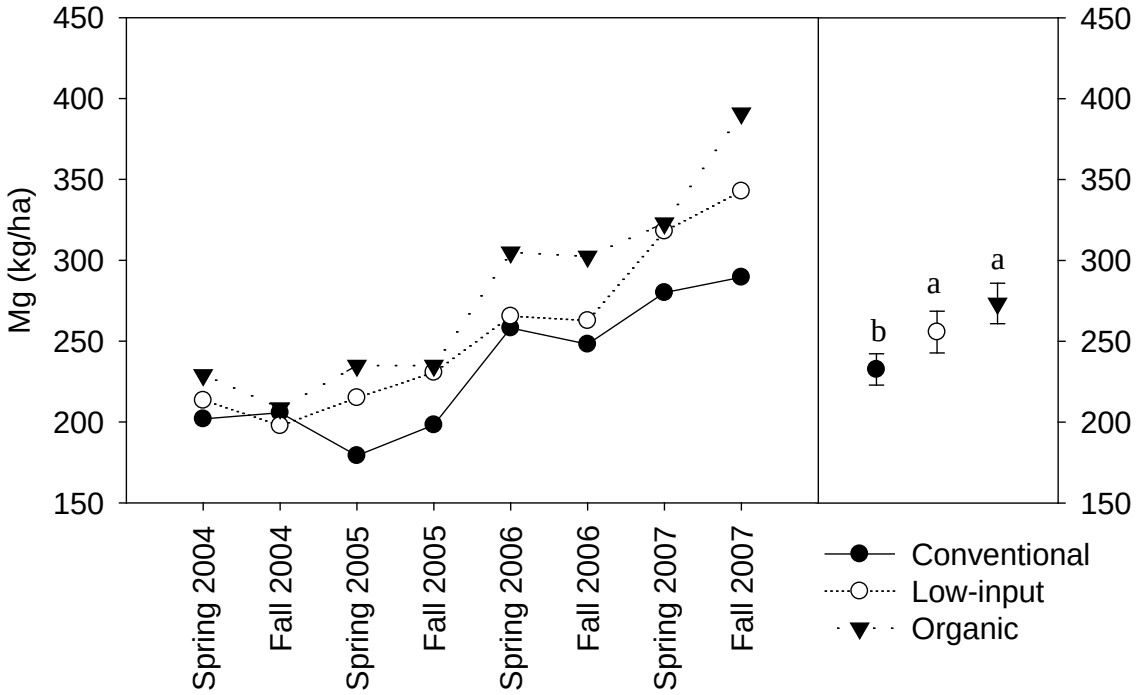


Figure 3.26. Mehlich III extracted Magnesium at 0-7 cm.
Symbols at right indicate the standard error of the grand mean for all sampling times. The presence of different lower-case letters denotes a significant difference using Tukeys HSD (LSMeans). Below are the results of the effect tests in a multi-way ANOVA. An asterisk indicates significance at the level of α = 0.05.

Source	DF	Mean Square	F Ratio	Prob > F
Treatment	2	17149.04	11.29	*<.0001
Block	3	4071.75	2.68	0.0536
Time	1	141569.05	93.17	*<.0001
Treatment*time	2	3133.74	2.06	0.1349
Error	69	1519.50		

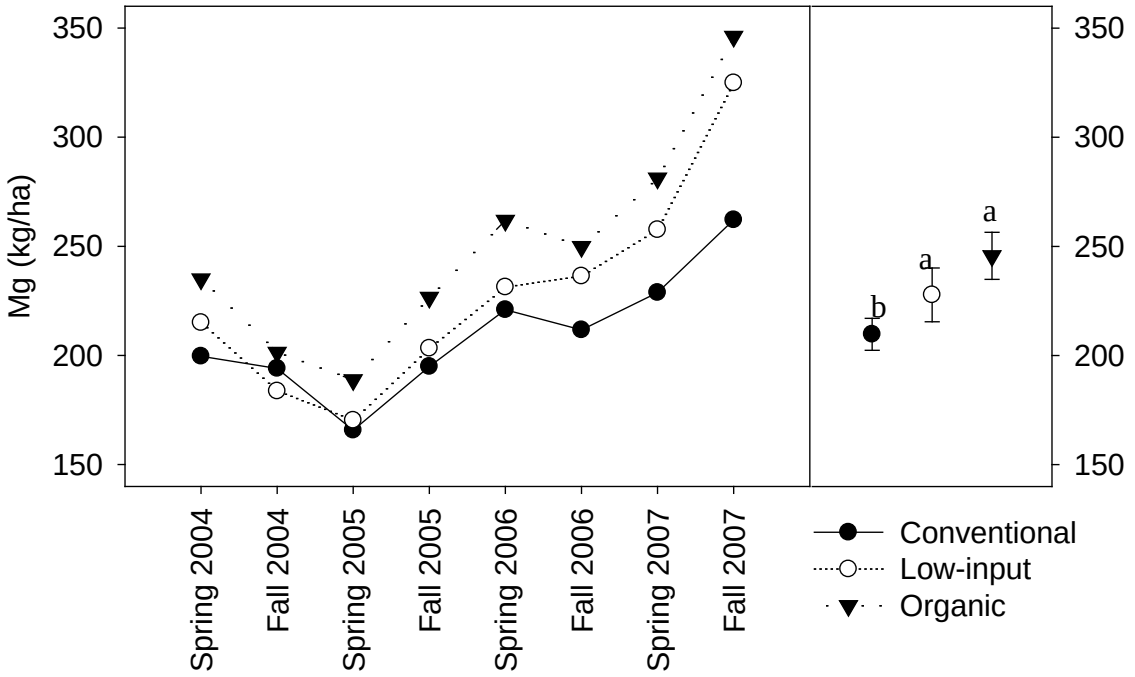


Figure 3.27. Mehlich III extracted Magnesium at 7-15 cm.
Symbols at right indicate the standard error of the grand mean for all sampling times. The presence of different lower-case letters denotes a significant difference using Tukeys HSD (LSMeans). Below are the results of the effect tests in a multi-way ANOVA. An asterisk indicates significance at the level of α = 0.05.

Source	DF	Mean Square	F Ratio	Prob > F
Treatment	2	14757.27	8.34	*0.0006
Block	3	5816.93	3.29	*0.0255
Time	1	75756.13	42.82	*<.0001
Treatment*time	2	3588.94	2.03	0.1391
Error	71	1769.00		

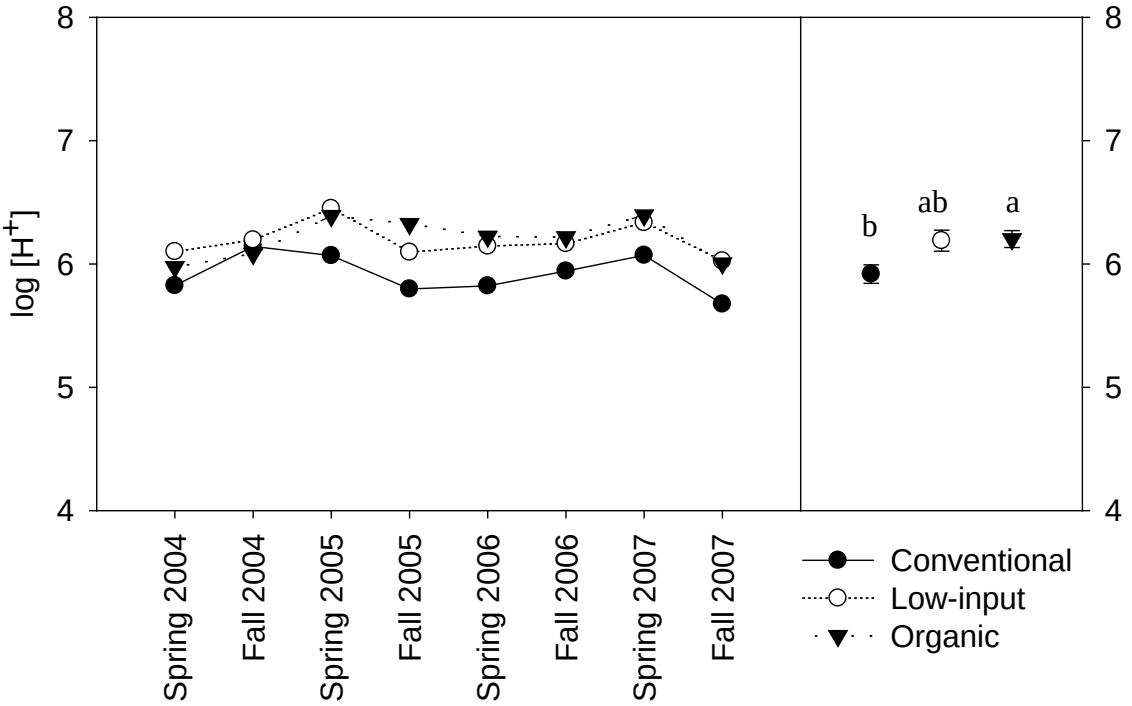


Figure 3.28. pH at 0-7 cm soil depth.

Symbols at right indicate the standard error of the grand mean for all sampling times. The presence of different lower-case letters denotes a significant difference using Tukeys HSD (LSMeans). Below are the results of the effect tests in a multi-way ANOVA. An asterisk indicates significance at the level of α = 0.05.

Source	DF	Mean Square	F Ratio	Prob > F
Treatment	2	0.64	4.55	*0.0139
Block	3	0.62	4.36	*0.0071
Time	1	0.00	0.00	0.9816
Treatment*time	2	0.33	0.41	0.6666
Error	71	0.14		

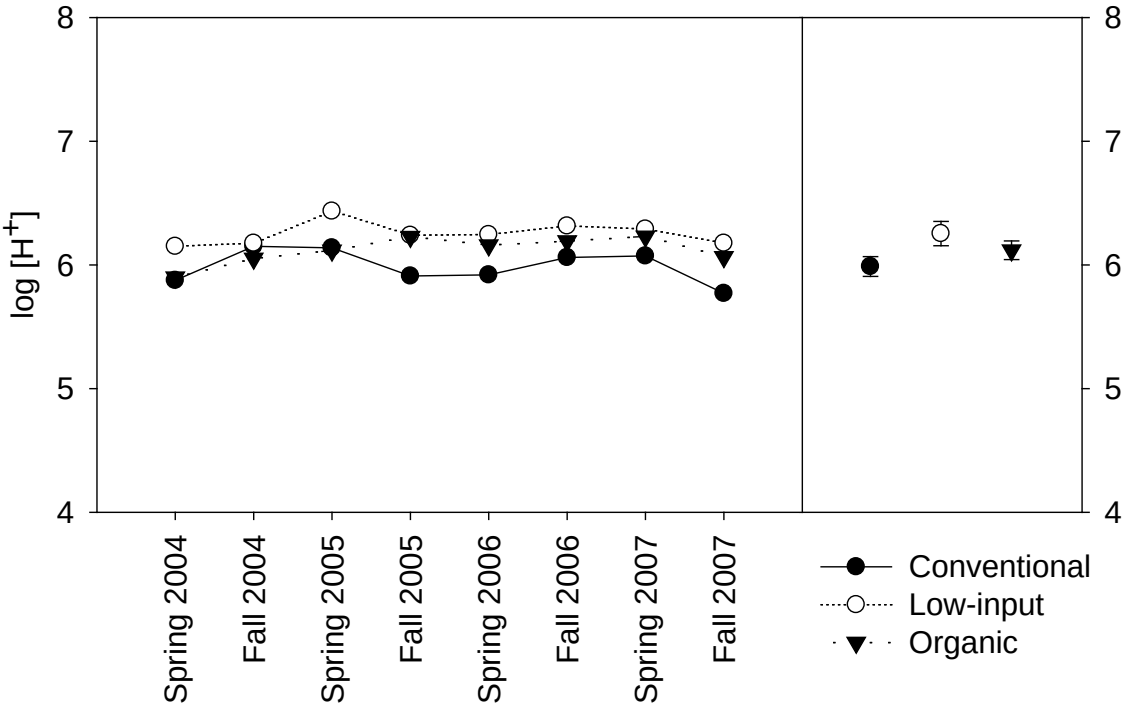


Figure 3.29. pH at 7-15 cm soil depth.

Symbols at right indicate the standard error of the grand mean for all sampling times. The presence of different lower-case letters denotes a significant difference using Tukeys HSD (LSMeans). Below are the results of the effect tests in a multi-way ANOVA. An asterisk indicates significance at the level of α = 0.05.

Source	DF	Mean Square	F Ratio	Prob > F
Treatment	2	0.14	0.97	0.3840
Block	3	1.27	8.72	*<.0001
Time	1	0.01	0.09	0.7652
Treatment*time	2	0.07	0.47	0.6288
Error	71	0.15		

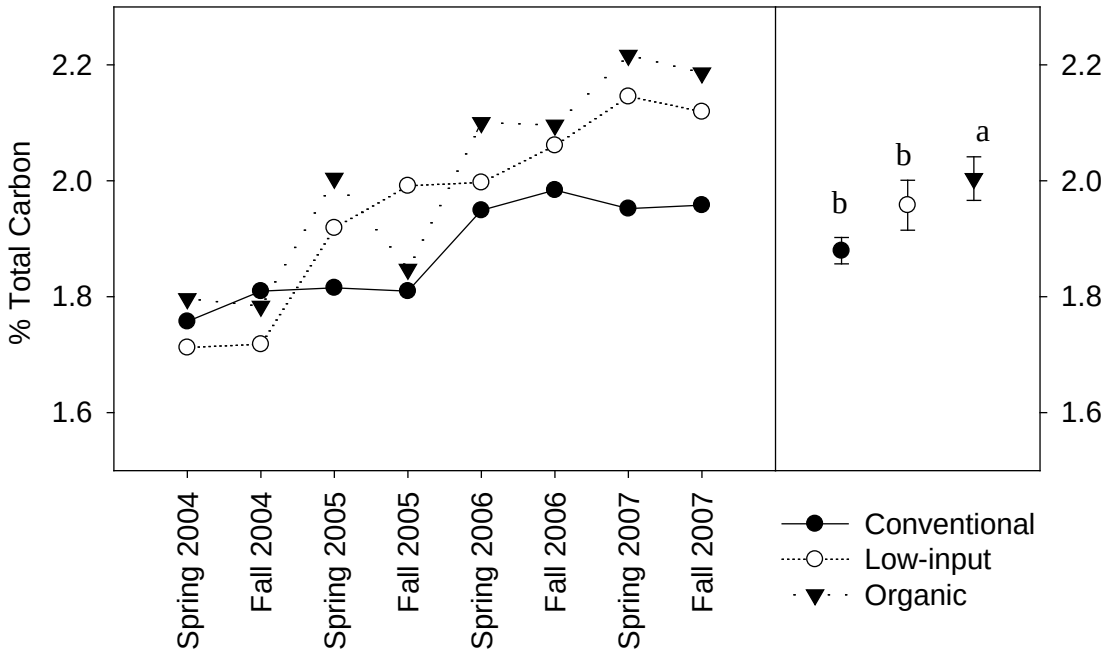


Figure 3.30. Percent total carbon in soil at 0-7 cm.
Symbols at right indicate the standard error of the grand mean for all sampling times. The presence of different lower-case letters denotes a significant difference using Tukeys HSD (LSMeans). Below are the results of the effect tests in a multi-way ANOVA. An asterisk indicates significance at the level of α = 0.05.

Source	DF	Mean Square	F Ratio	Prob > F
Treatment	2	0.12	17.48	*<.0001
Block	3	0.18	24.96	*<.0001
Time	1	1.16	162.36	*<.0001
Treatment*time	2	0.06	8.57	*0.0005
Error	71	0.01		

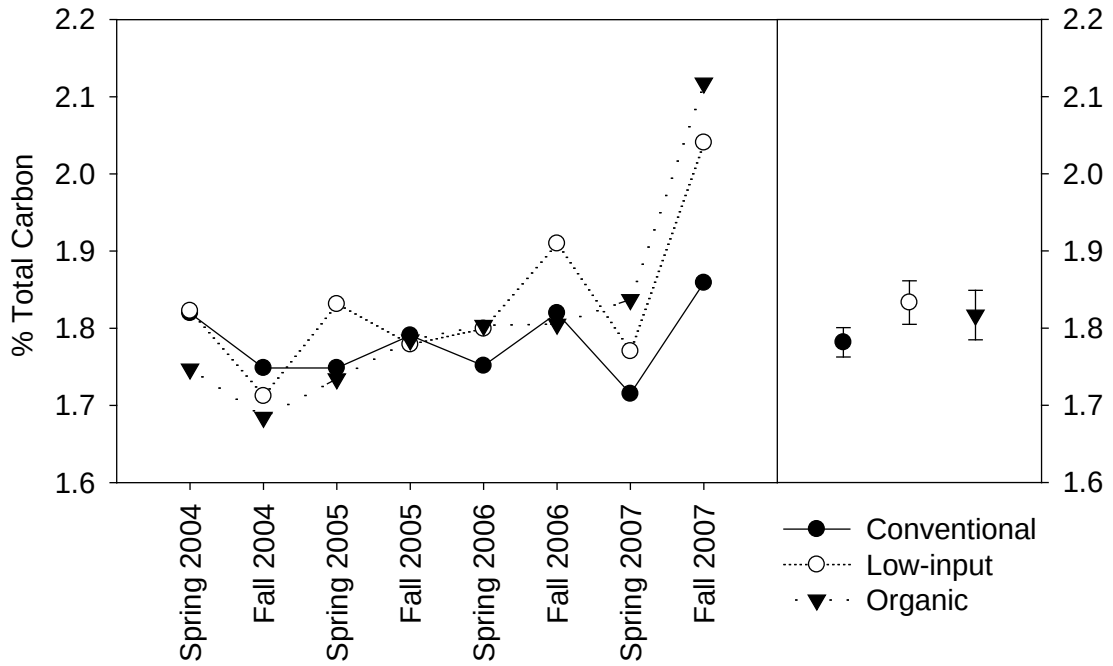


Figure 3.31. Percent total Carbon in soil at 7-15 cm.
Symbols at right indicate the standard error of the grand mean for all sampling times. The presence of different lower-case letters denotes a significant difference using Tukeys HSD (LSMeans). Below are the results of the effect tests in a multi-way ANOVA. An asterisk indicates significance at the level of α = 0.05.

Source	DF	Mean Square	F Ratio	Prob > F
Treatment	2	0.01	0.89	0.4139
Block	3	0.13	11.92	*<.0001
Time	1	0.17	15.15	*0.0002
Treatment*time	2	0.06	5.60	*0.0055
Error	70	0.01		

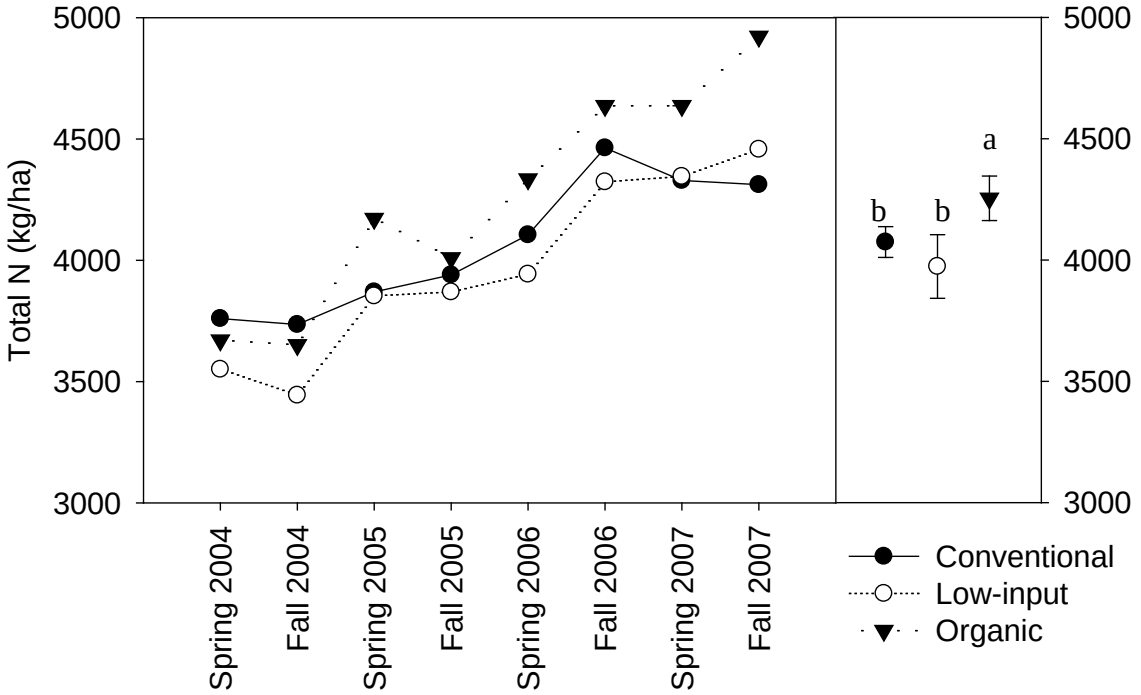


Figure 3.32. Total Nitrogen at 0-7 cm.

Symbols at right indicate the standard error of the grand mean for all sampling times. The presence of different lower-case letters denotes a significant difference using Tukeys HSD (LSMeans). Below are the results of the effect tests in a multi-way ANOVA. An asterisk indicates significance at the level of α = 0.05.

Source	DF	Mean Square	F Ratio	Prob > F
Treatment	2	300815.20	7.03	*0.0017
Block	3	1382559.33	32.31	*<.0001
Time	1	7668238.30	179.22	*<.0001
Treatment*time	2	266829.10	6.24	*0.0032
Error	70	42786.00		

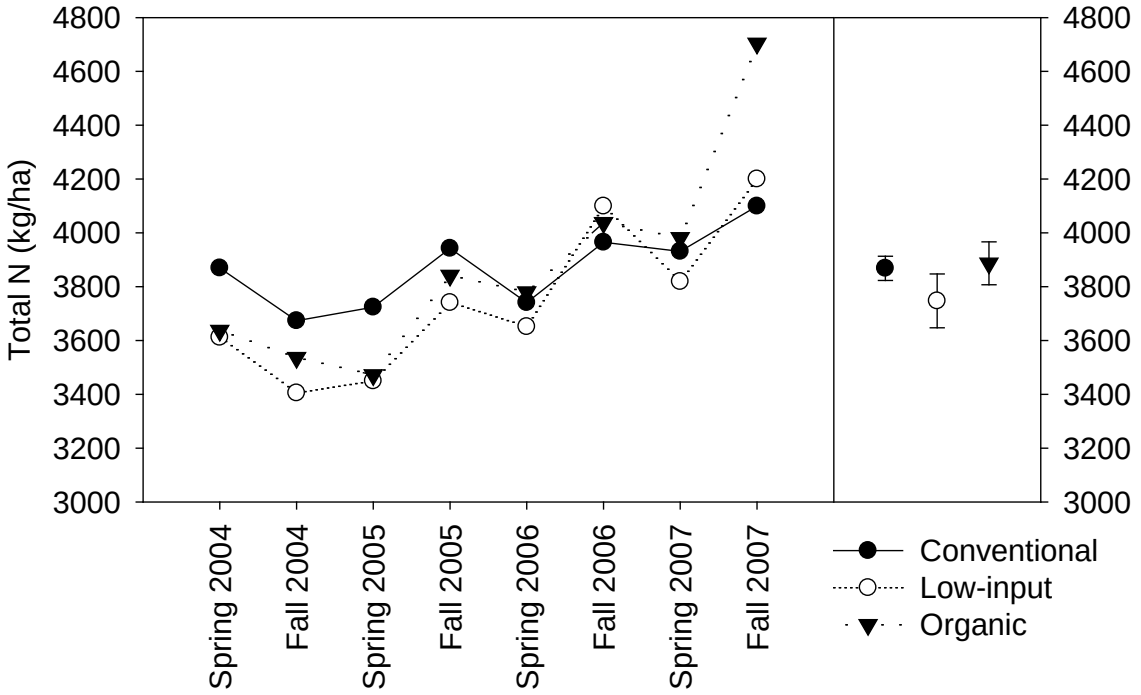


Figure 3.33. Total Nitrogen at 7-15 cm.

Symbols at right indicate the standard error of the grand mean for all sampling times. The presence of different lower-case letters denotes a significant difference using Tukeys HSD (LSMeans). Below are the results of the effect tests in a multi-way ANOVA. An asterisk indicates significance at the level of α = 0.05.

Source	DF	Mean Square	F Ratio	Prob > F
Treatment	2	10922.90	0.19	0.8297
Block	3	971504.23	16.65	*<.0001
Time	1	2476229.10	42.43	*<.0001
Treatment*time	2	289496.65	4.96	*0.0097
Error	70	58360.00		

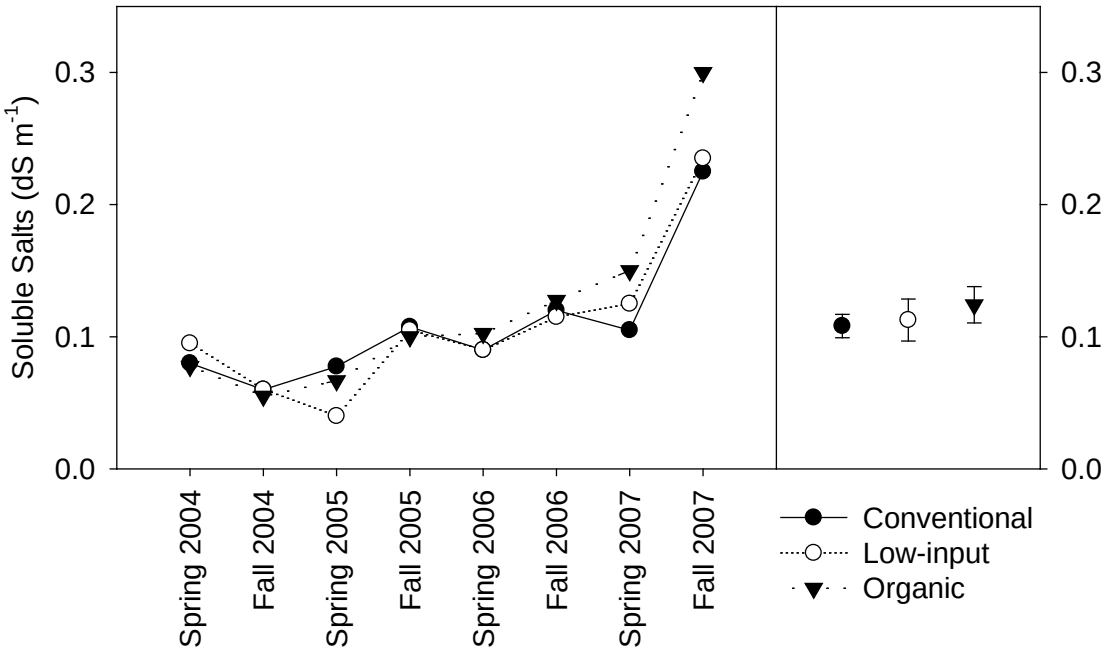


Figure 3.34. Soluble salts at 0-7 cm.

Symbols at right indicate the standard error of the grand mean for all sampling times. The presence of different lower-case letters denotes a significant difference using Tukeys HSD (LSMeans). Below are the results of the effect tests in a multi-way ANOVA. An asterisk indicates significance at the level of α = 0.05.

Source	DF	Mean Square	F Ratio	Prob > F
Treatment	2	0.00	0.92	0.402
Block	3	0.00	0.18	0.9121
Time	1	0.12	52.75	*<.0001
Treatment*time	2	0.01	2.27	0.111
Error	70	0.16	0.00	

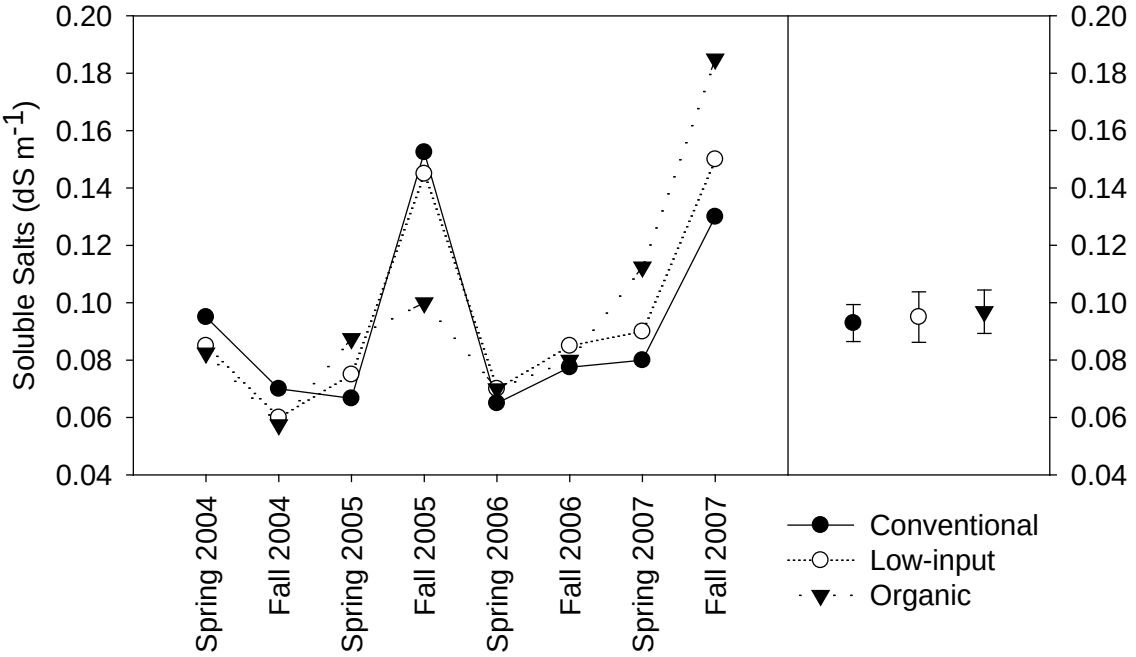


Figure 3.35. Soluble salts at 7-15 cm.

Symbols at right indicate the standard error of the grand mean for all sampling times. The presence of different lower-case letters denotes a significant difference using Tukeys HSD (LSMeans). Below are the results of the effect tests in a multi-way ANOVA. An asterisk indicates significance at the level of $\alpha = 0.05$.

Source	DF	Mean Square	F Ratio	Prob > F
Treatment	2	0.00	0.12	0.8913
Block	3	0.00	0.26	0.8529
Time	1	0.01	9.55	*0.0029
Treatment*time	2	0.00	2.80	0.0674
Error	70	0.00		

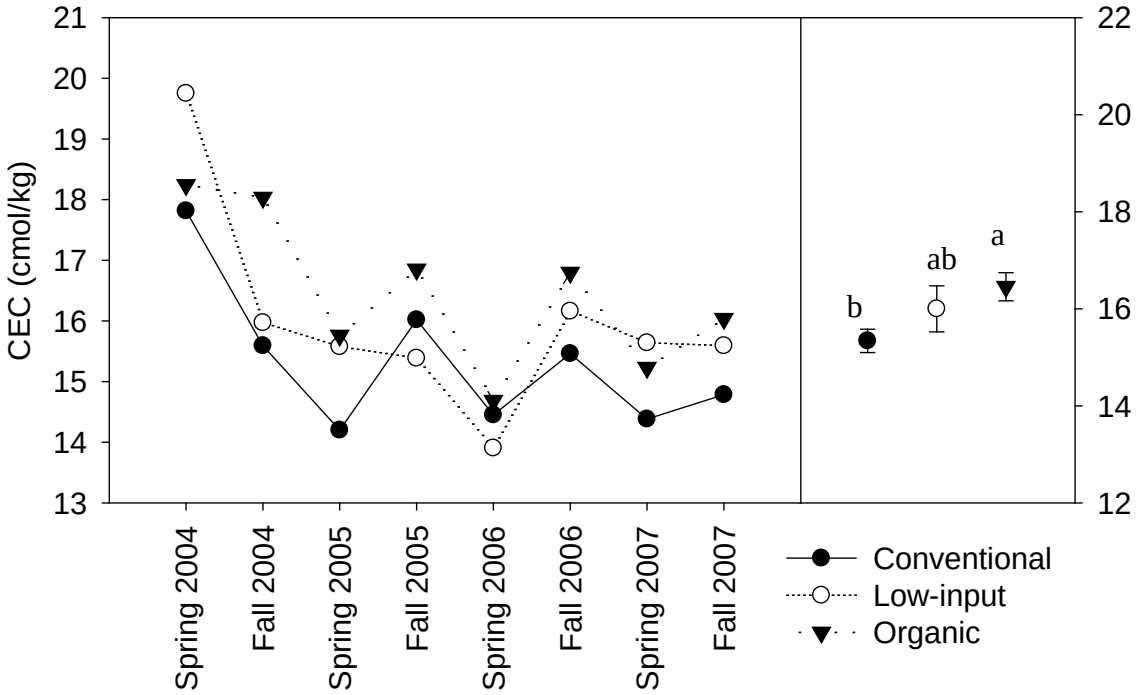


Figure 3.36. Cation exchange capacity at 0-7 cm.
Symbols at right indicate the standard error of the grand mean for all sampling times. The presence of different lower-case letters denotes a significant difference using Tukeys HSD (LSMeans). Below are the results of the effect tests in a multi-way ANOVA. An asterisk indicates significance at the level of α = 0.05.

Source	DF	Mean Square	F Ratio	Prob > F
Treatment	2	9.94	5.54	*0.0058
Block	3	2.25	1.25	0.2968
Time	1	48.21	26.88	*<.0001
Treatment*time	2	0.30	0.16	0.8484
Error	70	125.55	1.79	

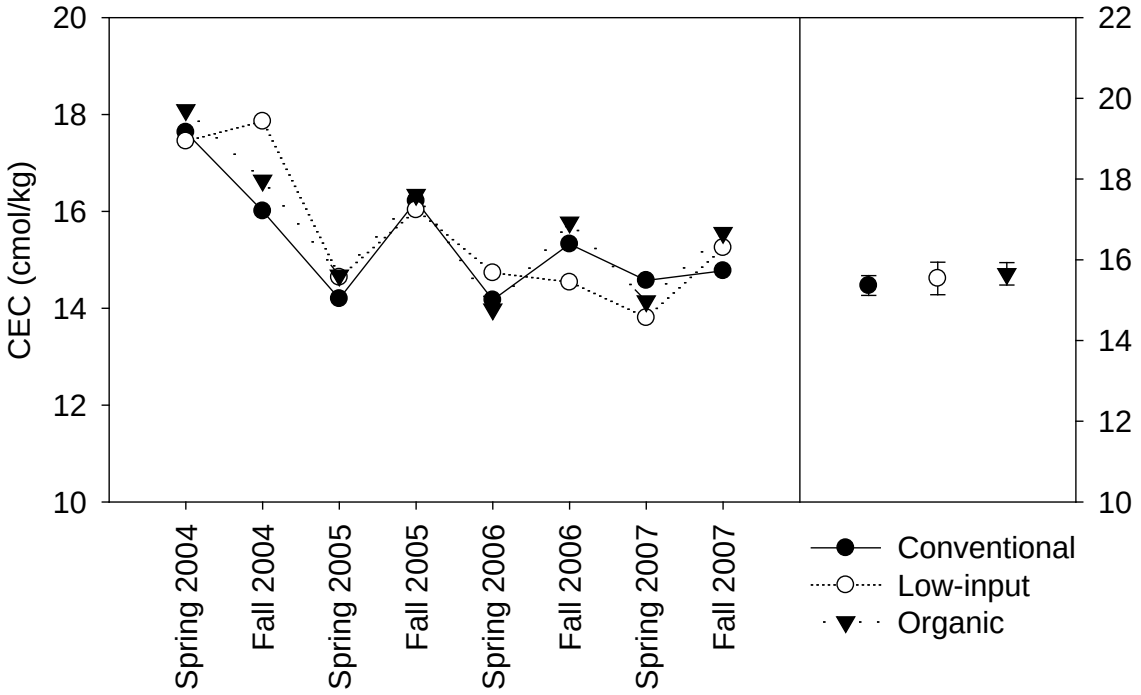


Figure 3.37. Cation exchange capacity at 7-15 cm.
Symbols at right indicate the standard error of the grand mean for all sampling times. The presence of different lower-case letters denotes a significant difference using Tukeys HSD (LSMeans). Below are the results of the effect tests in a multi-way ANOVA. An asterisk indicates significance at the level of α = 0.05.

Source	DF	Mean Square	F Ratio	Prob > F
Treatment	2	0.68	0.45	0.6387
Block	3	0.67	0.45	0.7209
Time	1	63.30	42.19	*<.0001
Treatment*time	2	0.68	0.45	0.6367
Error	71	1.50		

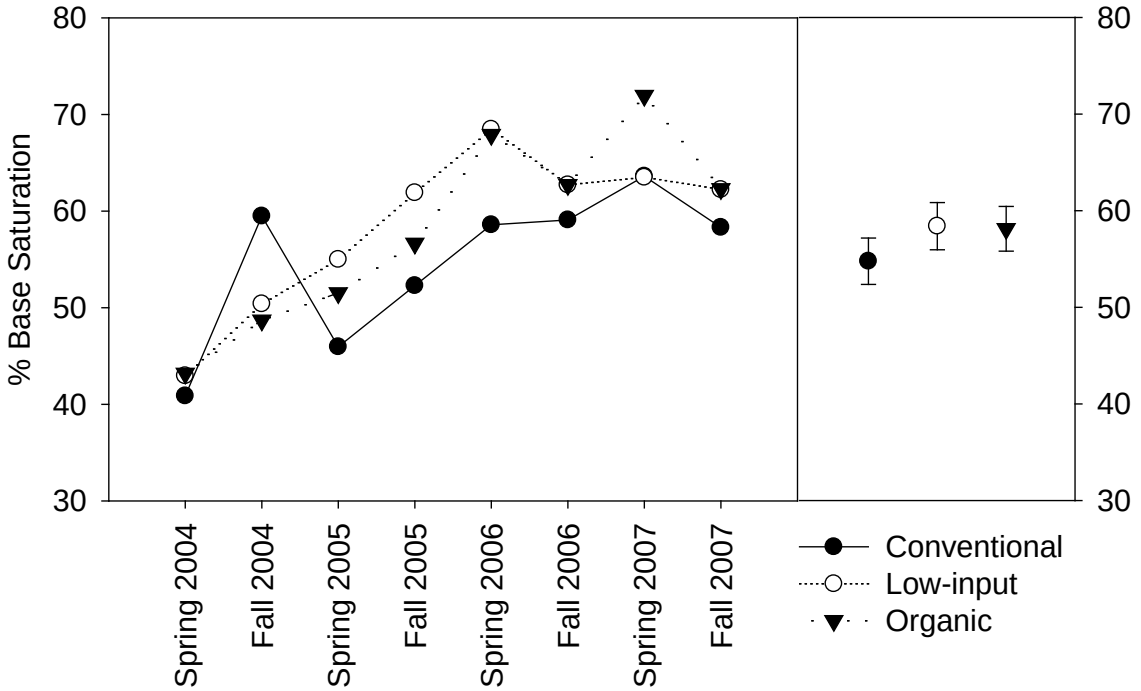


Figure 3.38. Percent base saturation at 0-7 cm.

Symbols at right indicate the standard error of the grand mean for all sampling times. The presence of different lower-case letters denotes a significant difference using Tukeys HSD (LSMeans). Below are the results of the effect tests in a multi-way ANOVA. An asterisk indicates significance at the level of α = 0.05.

Source	DF	Mean Square	F Ratio	Prob > F
Treatment	2	183.85	0.83	0.4393
Block	3	1065.90	3.22	*0.0279
Time	1	2976.14	26.94	*<.0001
Treatment*time	2	214.55	0.97	0.3836
Error	71	7842.20	110.45	

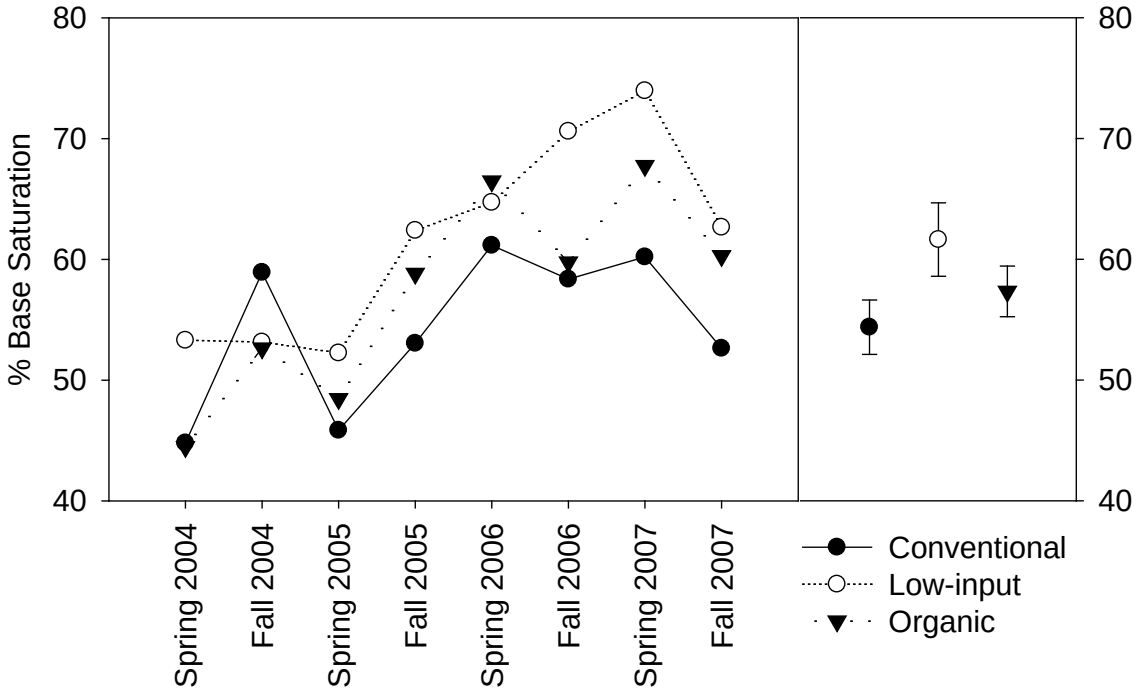


Figure 3.39. Percent base saturation at 7-15 cm.

Symbols at right indicate the standard error of the grand mean for all sampling times. The presence of different lower-case letters denotes a significant difference using Tukeys HSD (LSMeans). Below are the results of the effect tests in a multi-way ANOVA. An asterisk indicates significance at the level of α = 0.05.

Source	DF	Mean Square	F Ratio	Prob > F
Treatment	2	120.09	1.06	0.3532
Block	3	479.66	4.22	*0.0084
Time	1	1834.47	16.13	*0.0001
Treatment*time	2	121.39	1.07	0.3494
Error	71	113.72		

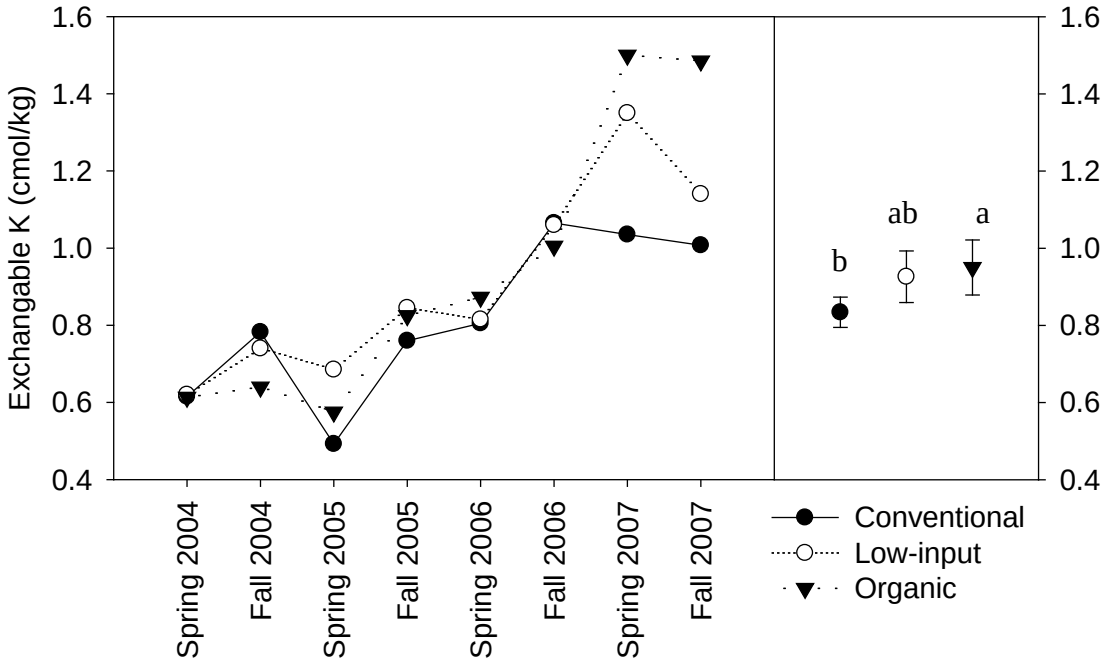


Figure 3.40. Exchangeable potassium at 0-7 cm.

Symbols at right indicate the standard error of the grand mean for all sampling times. The presence of different lower-case letters denotes a significant difference using Tukeys HSD (LSMeans). Below are the results of the effect tests in a multi-way ANOVA. An asterisk indicates significance at the level of α = 0.05.

Source	DF	Mean Square	F Ratio	Prob > F
Treatment	2	0.13	3.27	*0.0443
Block	3	0.05	1.19	0.3199
Time	1	3.12	80.65	*<.0001
Treatment*time	2	0.24	6.11	*0.0036
Error	67	0.04		

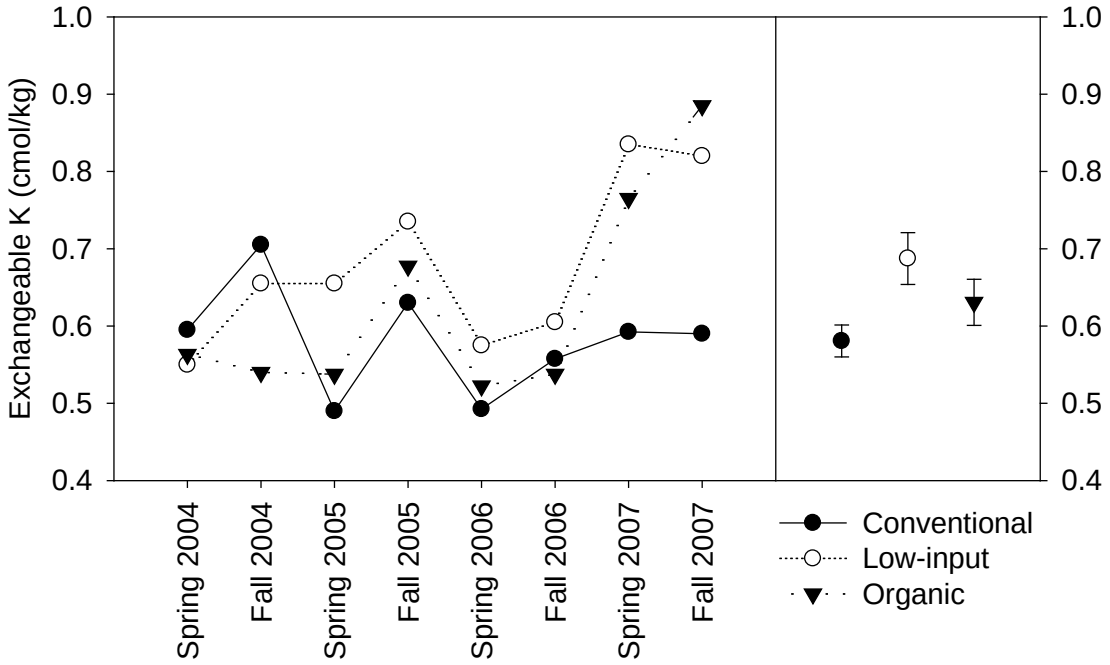


Figure 3.41. Exchangeable potassium at 7-15 cm.
Symbols at right indicate the standard error of the grand mean for all sampling times. The presence of different lower-case letters denotes a significant difference using Tukeys HSD (LSMeans). Below are the results of the effect tests in a multi-way ANOVA. An asterisk indicates significance at the level of α = 0.05.

Source	DF	Mean Square	F Ratio	Prob > F
Treatment	2	0.03	2.03	0.1388
Block	3	0.06	4.14	*0.0095
Time	1	0.08	5.74	*0.0194
Treatment*time	2	0.09	6.24	*0.0033
Error	67	0.01		

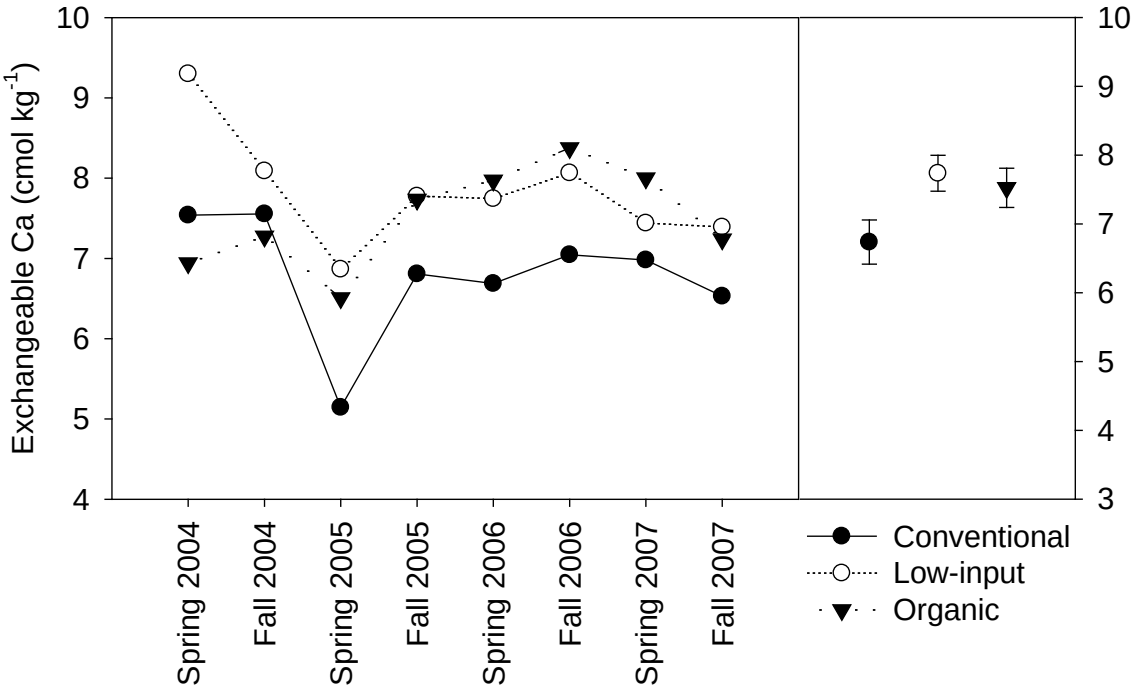


Figure 3.42. Exchangeable calcium at 0-7 cm.

Symbols at right indicate the standard error of the grand mean for all sampling times. The presence of different lower-case letters denotes a significant difference using Tukeys HSD (LSMeans). Below are the results of the effect tests in a multi-way ANOVA. An asterisk indicates significance at the level of α = 0.05.

Source	DF	Mean Square	F Ratio	Prob > F
Treatment	2	5.39	2.27	0.1112
Block	3	5.60	2.36	0.0794
Time	1	0.01	0.00	0.9503
Treatment*time	2	1.58	0.66	0.5184
Error	67	2.38		

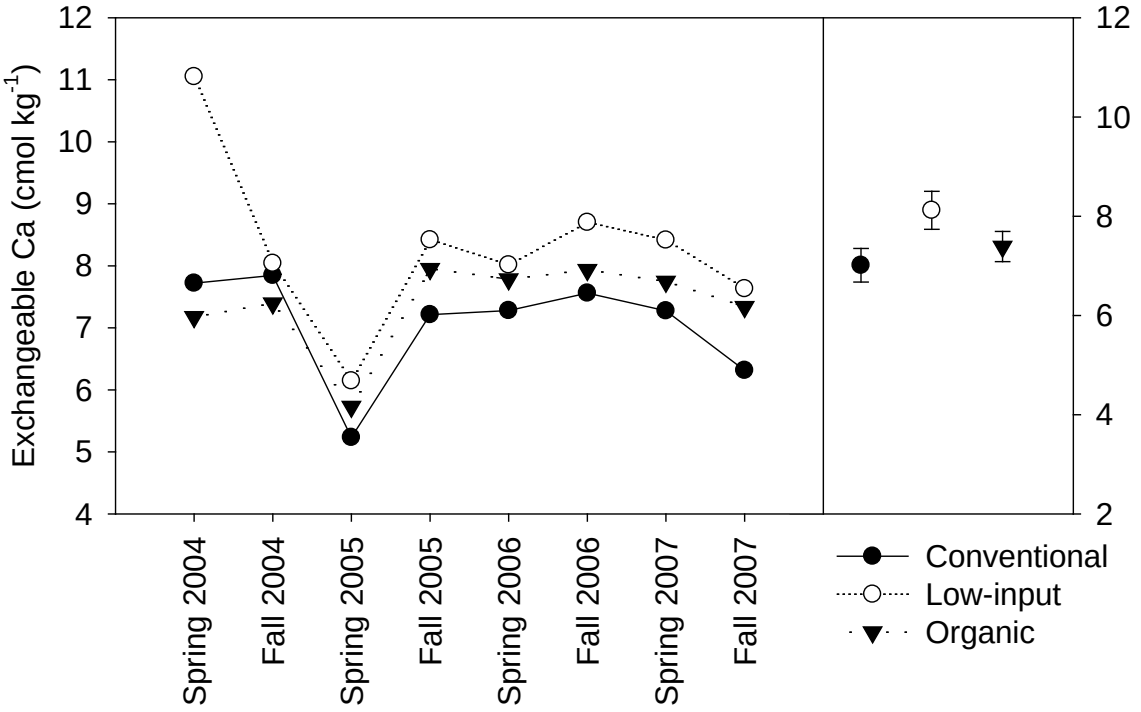


Figure 3.43. Exchangeable calcium at 7-15 cm.

Symbols at right indicate the standard error of the grand mean for all sampling times. The presence of different lower-case letters denotes a significant difference using Tukeys HSD (LSMeans). Below are the results of the effect tests in a multi-way ANOVA. An asterisk indicates significance at the level of α = 0.05.

Source	DF	Mean Square	F Ratio	Prob > F
Treatment	2	2.10	0.76	0.4698
Block	3	10.08	3.66	*0.0165
Time	1	0.01	0.00	0.9532
Treatment*time	2	0.86	0.31	0.7316
Error	67	2.75		

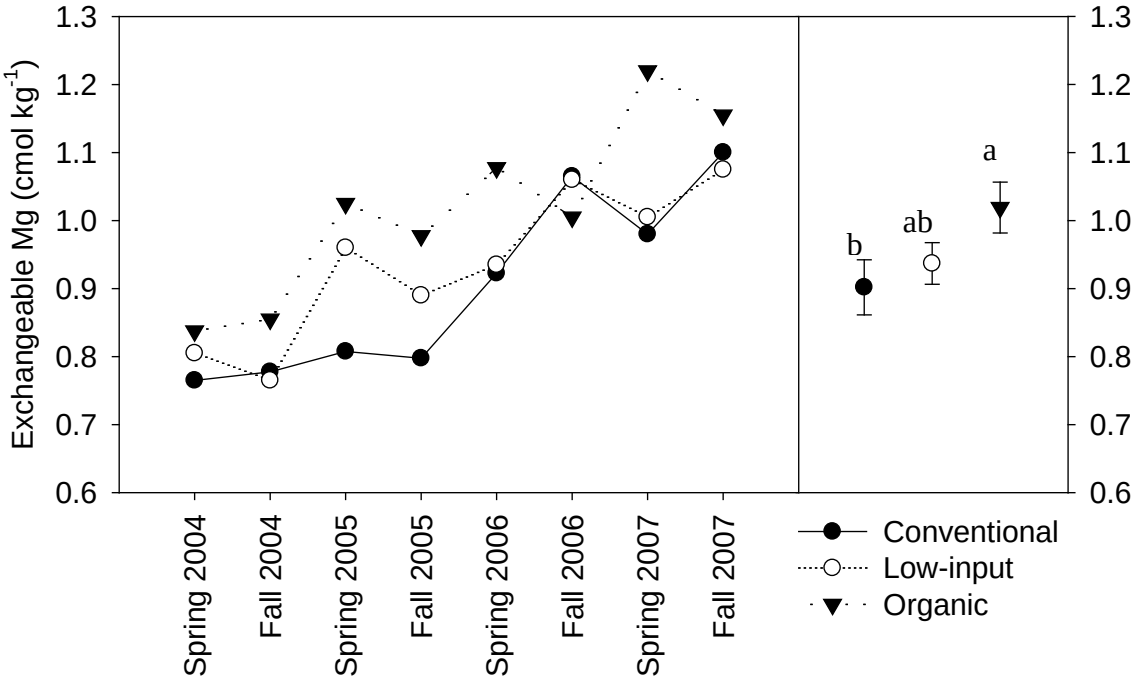


Figure 3.44. Exchangeable magnesium at 0-7 cm.

Symbols at right indicate the standard error of the grand mean for all sampling times. The presence of different lower-case letters denotes a significant difference using Tukeys HSD (LSMeans). Below are the results of the effect tests in a multi-way ANOVA. An asterisk indicates significance at the level of α = 0.05.

Source	DF	Mean Square	F Ratio	Prob > F
Treatment	2	0.18	6.64	*0.0023
Block	3	0.11	3.99	*0.0111
Time	1	0.82	29.82	*<.0001
Treatment*time	2	0.01	0.29	0.7464
Error	71	0.03		

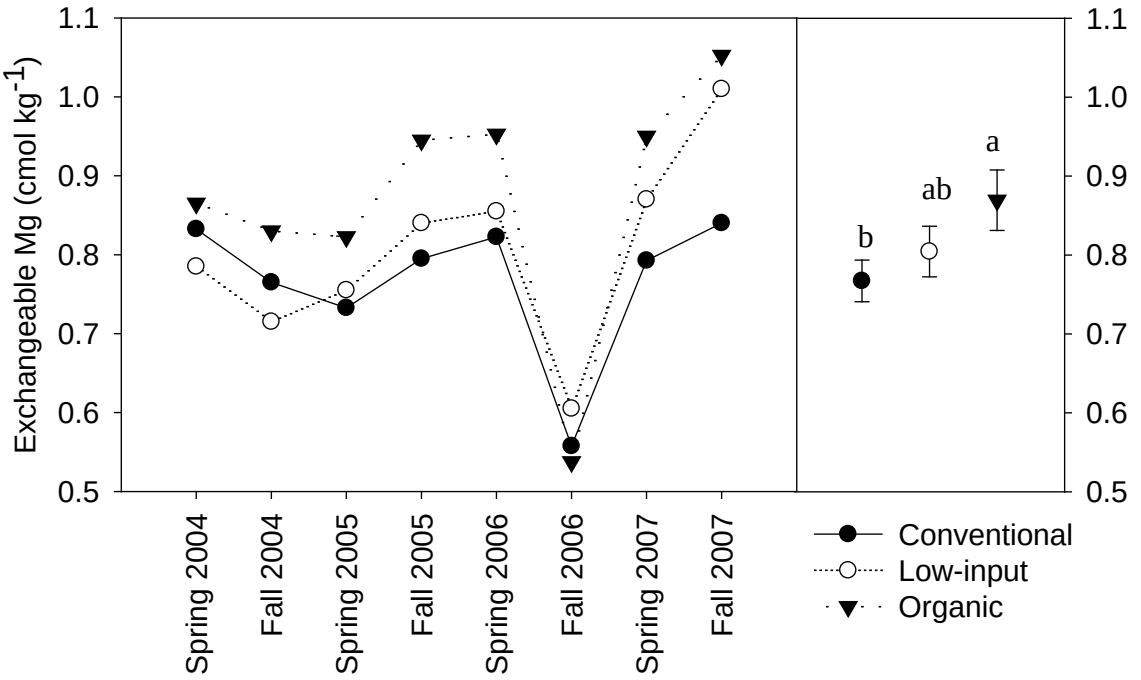


Figure 3.45. Exchangeable magnesium at 7-15 cm.

Symbols at right indicate the standard error of the grand mean for all sampling times. The presence of different lower-case letters denotes a significant difference using Tukeys HSD (LSMeans). Below are the results of the effect tests in a multi-way ANOVA. An asterisk indicates significance at the level of α = 0.05.

Source	DF	Mean Square	F Ratio	Prob > F
Treatment	2	0.12	6.89	*0.0018
Block	3	0.07	3.83	*0.0134
Time	1	0.16	8.95	*0.0038
Treatment*time	2	0.03	1.47	0.2367
Error	71	0.02		

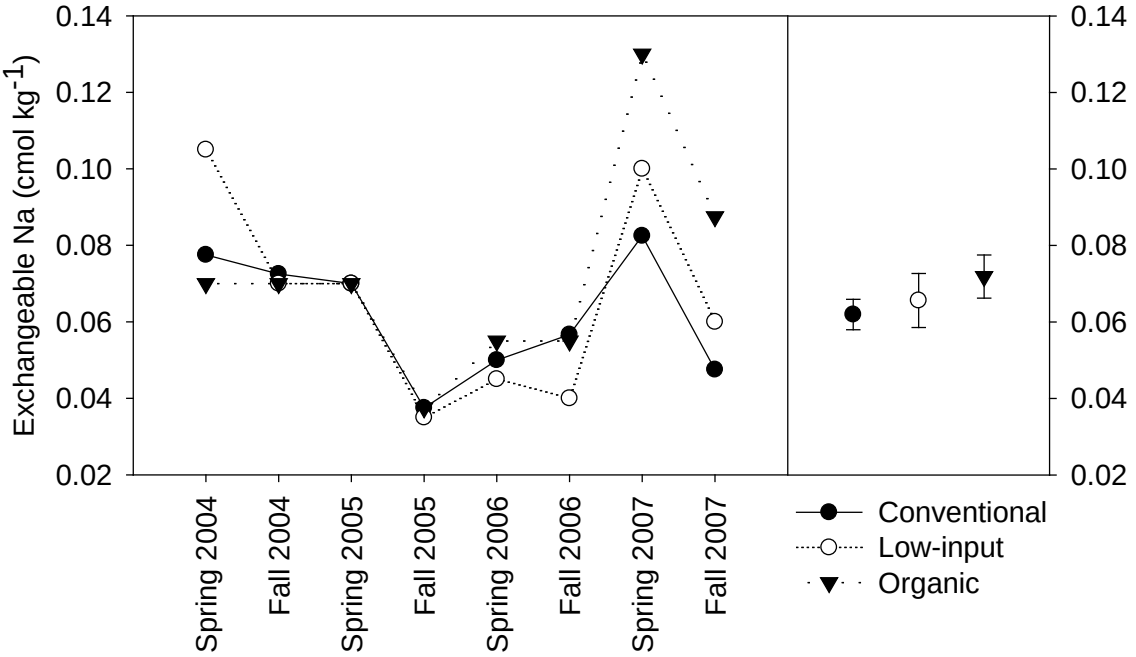


Figure 3.46. Exchangeable sodium at 0-7 cm.

Symbols at right indicate the standard error of the grand mean for all sampling times. The presence of different lower-case letters denotes a significant difference using Tukeys HSD (LSMeans). Below are the results of the effect tests in a multi-way ANOVA. An asterisk indicates significance at the level of α = 0.05.

Source	DF	Mean Square	F Ratio	Prob > F
Treatment	2	0.00	1.18	0.3138
Block	3	0.00	0.60	0.6158
Time	1	0.00	0.41	0.5250
Treatment*time	2	0.00	3.65	*0.0310
Error	70	0.00		

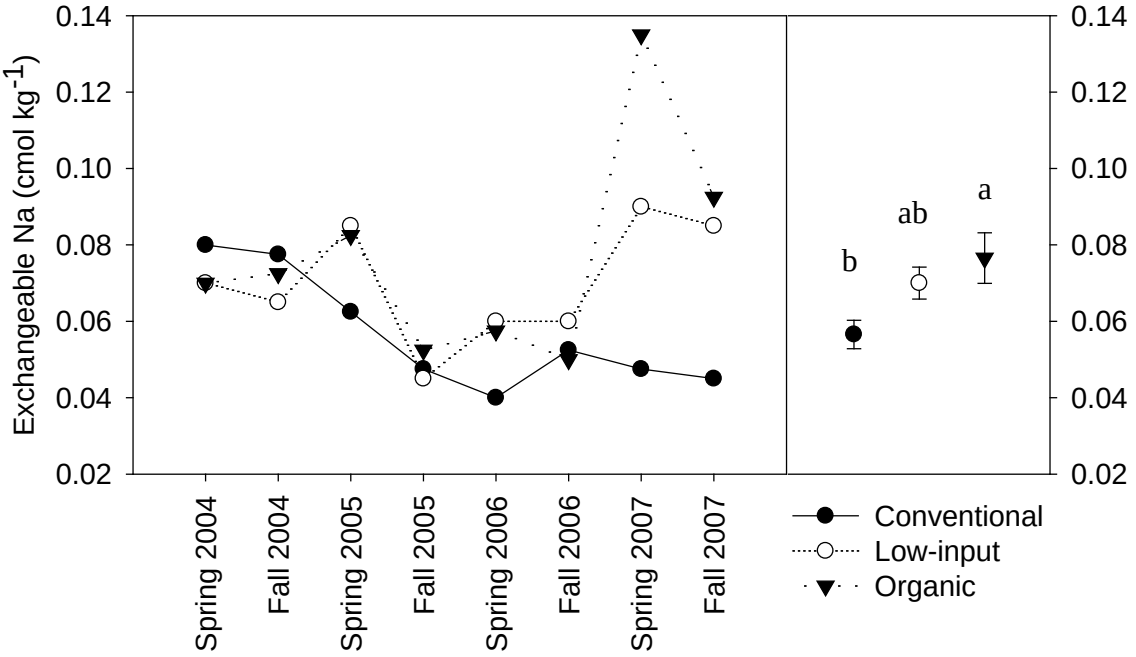


Figure 3.47. Exchangeable sodium at 7-15 cm.

Symbols at right indicate the standard error of the grand mean for all sampling times. The presence of different lower-case letters denotes a significant difference using Tukeys HSD (LSMeans). Below are the results of the effect tests in a multi-way ANOVA. An asterisk indicates significance at the level of α = 0.05.

Source	DF	Mean Square	F Ratio	Prob > F
Treatment	2	0.00	4.51	*0.0144
Block	3	0.00	0.39	0.7609
Time	1	0.00	0.00	0.9789
Treatment*time	2	0.00	7.82	*0.0009
Error	70	0.00		

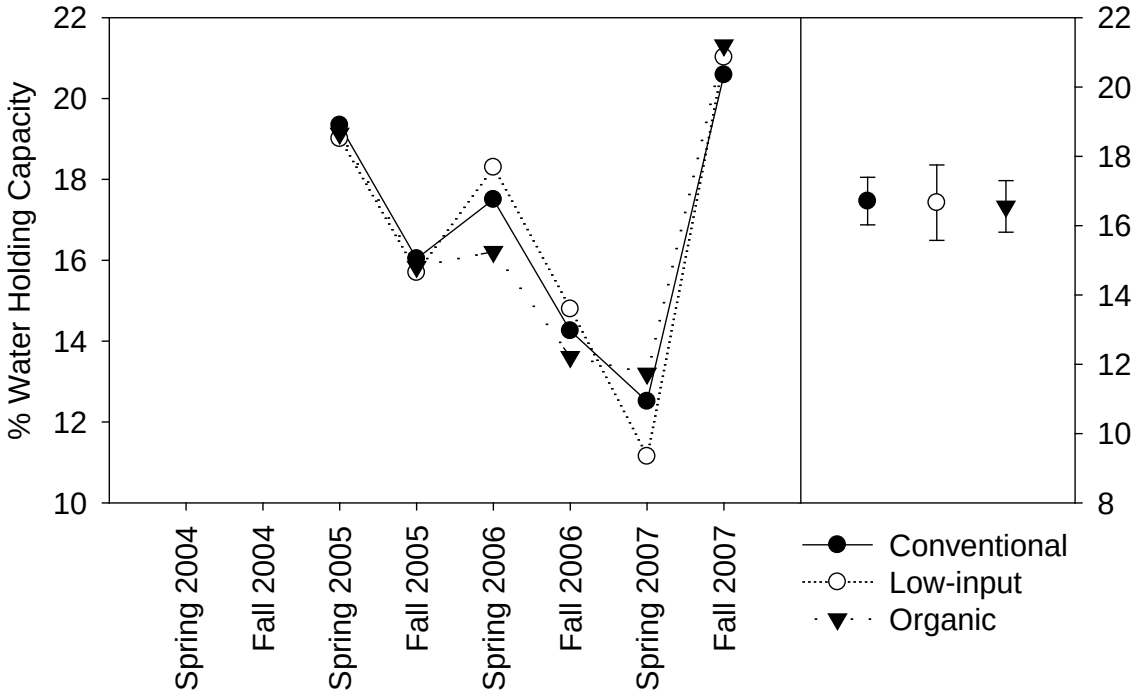


Figure 3.48. Water holding capacity, 0-7 cm soil depth.
Symbols at right indicate the standard error of the grand mean for all sampling times (data not available for 2004). The presence of different lower-case letters denotes a significant difference using Tukeys HSD (LSMeans). Below are the results of the effect tests in a multi-way ANOVA. An asterisk indicates significance at the level of $\alpha = 0.05$.

Source	DF	Mean Square	F Ratio	Prob > F
Treatment	2	1.40	0.10	0.9022
Block	3	6.60	0.49	0.6934
Time	1	6.96	0.51	0.4772
Treatment*time	2	1.11	0.08	0.9216
Error	51	13.58		

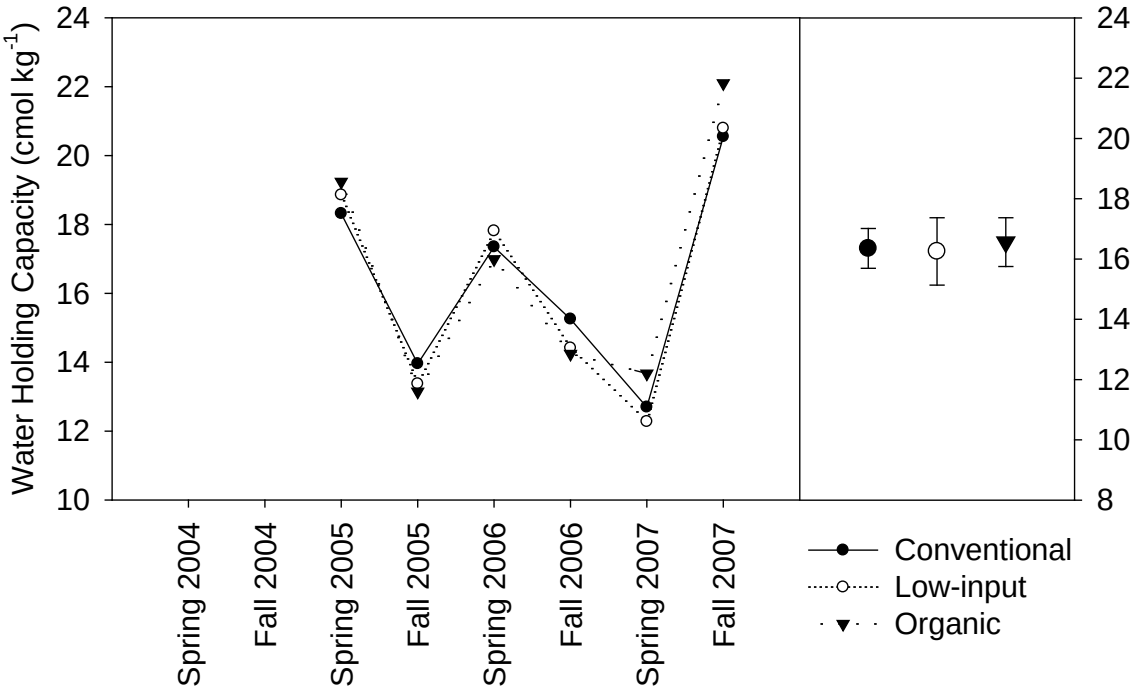


Figure 3.49. Water holding capacity, 7-15 cm.

Symbols at right indicate the standard error of the grand mean for all sampling times (data not available for 2004). The presence of different lower-case letters denotes a significant difference using Tukeys HSD (LSMeans). Below are the results of the effect tests in a multi-way ANOVA. An asterisk indicates significance at the level of α = 0.05.

Source	DF	Mean Square	F Ratio	Prob > F
Treatment	2	0.69	0.05	0.9531
Block	3	6.45	0.45	0.7205
Time	1	6.77	0.47	0.4967
Treatment*time	2	1.81	0.13	0.8825
Error	51	14.44		

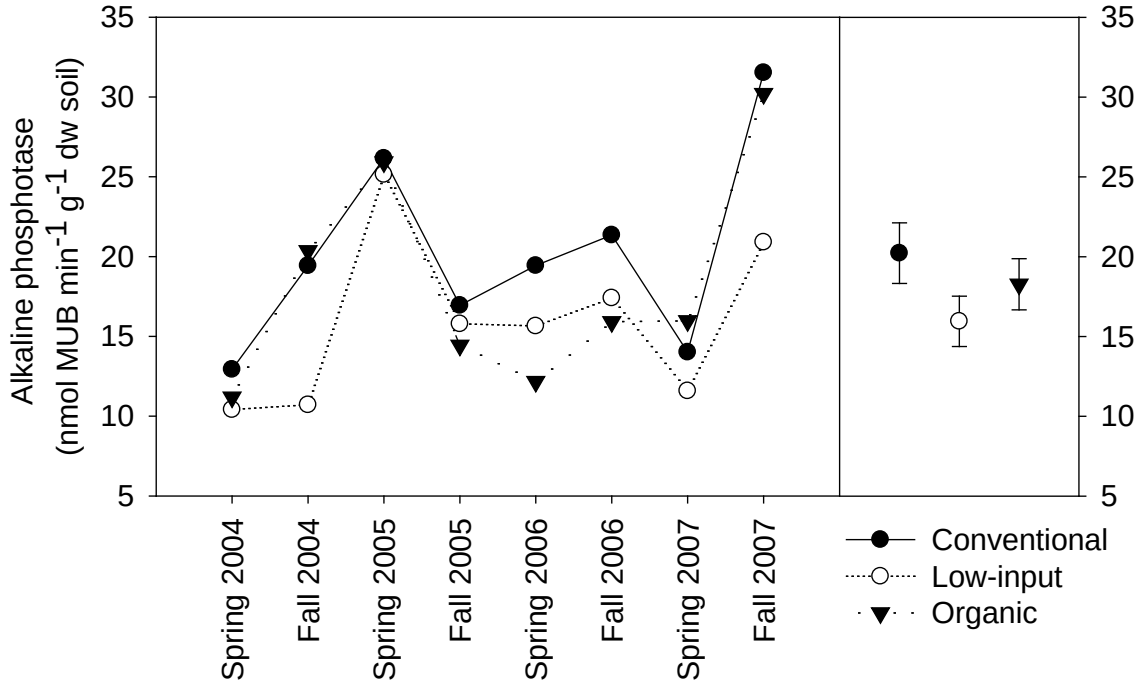


Figure 3.50. Alkaline phosphatase maximum activity under substrate saturation, 0-7 cm.

Symbols at right indicate the standard error of the grand mean for all sampling times. The presence of different lower-case letters denotes a significant difference using Tukeys HSD (LSMeans). Below are the results of the effect tests in a multi-way ANOVA. An asterisk indicates significance at the level of α = 0.05.

Source	DF	Mean Square	F Ratio	Prob > F
Treatment	2	0.07	0.45	0.6377
Block	3	1.56	10.14	*<.0001
Time	1	0.68	4.45	0.0385
Treatment*time	2	0.00	0.00	0.9974
Error	71	10.92	0.15	

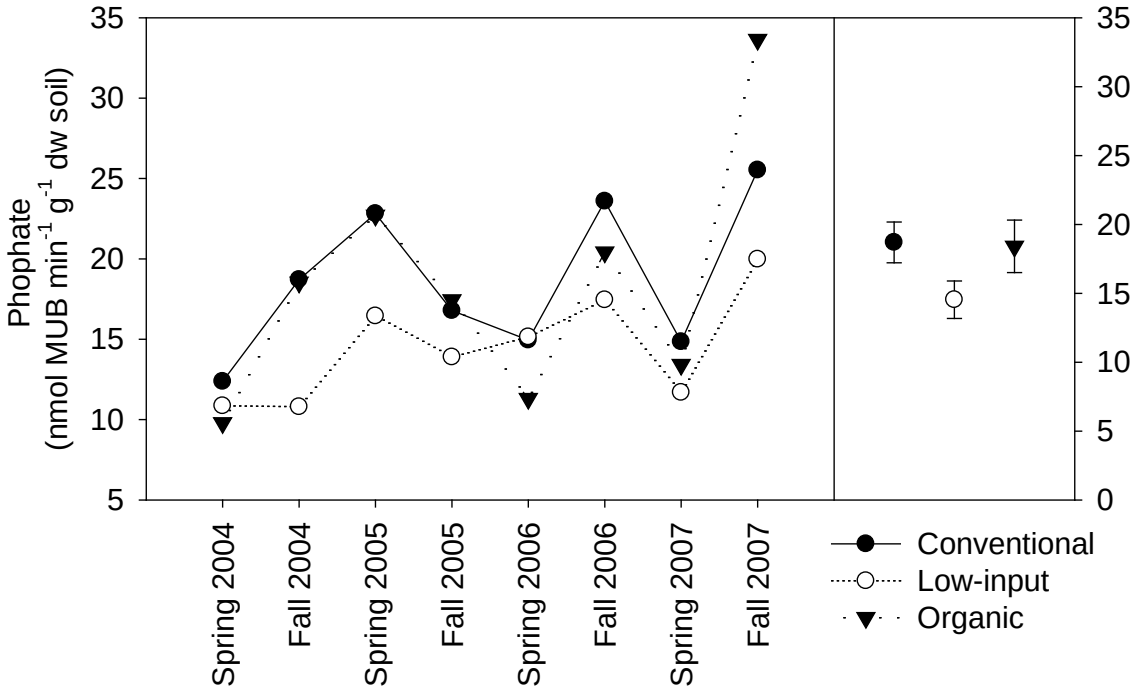


Figure 3.51. Alkaline phosphatase maximum activity under substrate saturation, at 7-15 cm soil depth.

Symbols at right indicate the standard error of the grand mean for all sampling times. The presence of different lower-case letters denotes a significant difference using Tukeys HSD (LSMeans). Below are the results of the effect tests in a multi-way ANOVA. An asterisk indicates significance at the level of α = 0.05.

Source	DF	Mean Square	F Ratio	Prob > F
Treatment	2	0.03	0.23	0.7967
Block	3	1.43	10.27	*<.0001
Time	1	0.94	6.72	0.0115
Treatment*time	2	0.01	0.08	0.9216
Error	71	9.900598	0.139445	

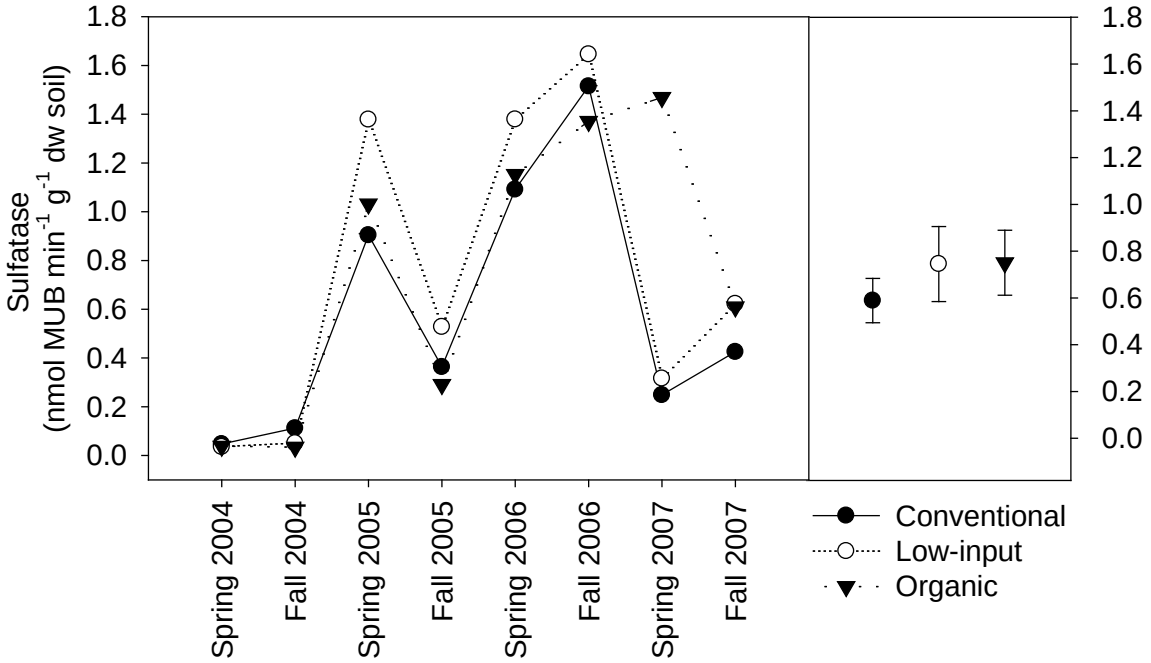


Figure 3.52. Sulfatase maximum activity under substrate saturation, at 0-7 cm soil depth.

Symbols at right indicate the standard error of the grand mean for all sampling times. The presence of different lower-case letters denotes a significant difference using Tukeys HSD (LSMeans). Below are the results of the effect tests in a multi-way ANOVA. An asterisk indicates significance at the level of α = 0.05.

Source	DF	Mean Square	F Ratio	Prob > F
Treatment	2	0.05	0.44	0.6464
Block	3	0.08	0.73	0.5384
Time	1	1.85	17.83	*<.0001
Treatment*time	2	0.13	1.29	0.2805
Error	71	7.36	0.10	

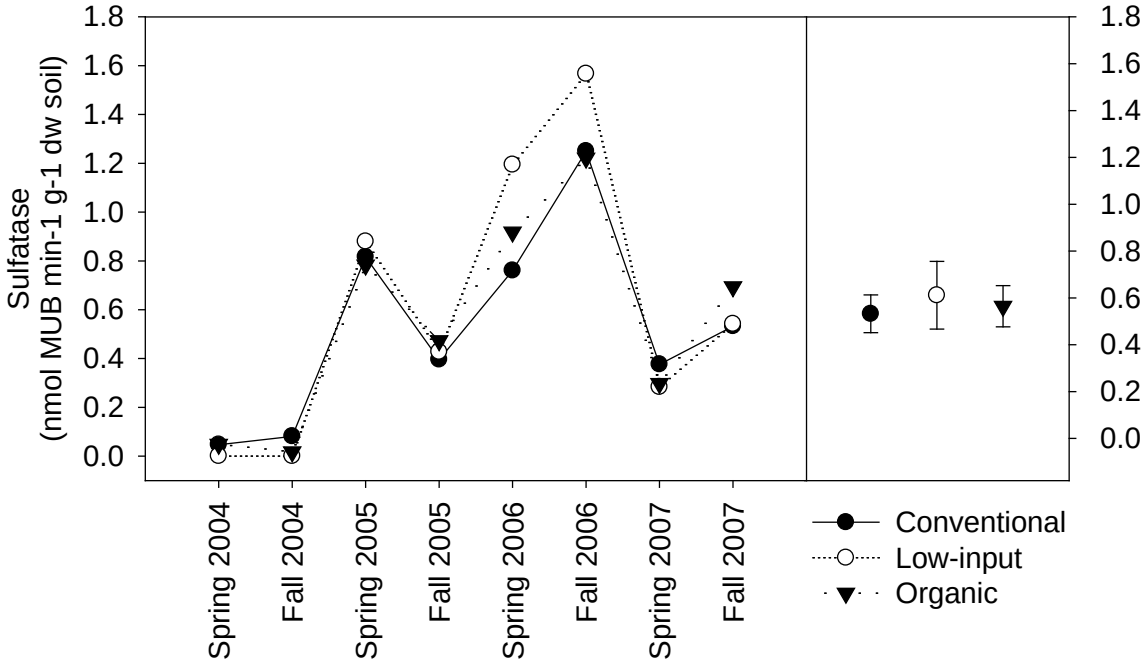


Figure 3.53. Sulfatase maximum activity under substrate saturation, at 7-15 cm soil depth.

Symbols at right indicate the standard error of the grand mean for all sampling times. The presence of different lower-case letters denotes a significant difference using Tukeys HSD (LSMeans). Below are the results of the effect tests in a multi-way ANOVA. An asterisk indicates significance at the level of α = 0.05.

Source	DF	Mean Square	F Ratio	Prob > F
Treatment	2	0.00	0.07	0.935
Block	3	0.07	0.89	0.4526
Time	1	1.49	20.29	*<.0001
Treatment*time	2	0.01	0.13	0.8745
Error	70	5.14	0.07	

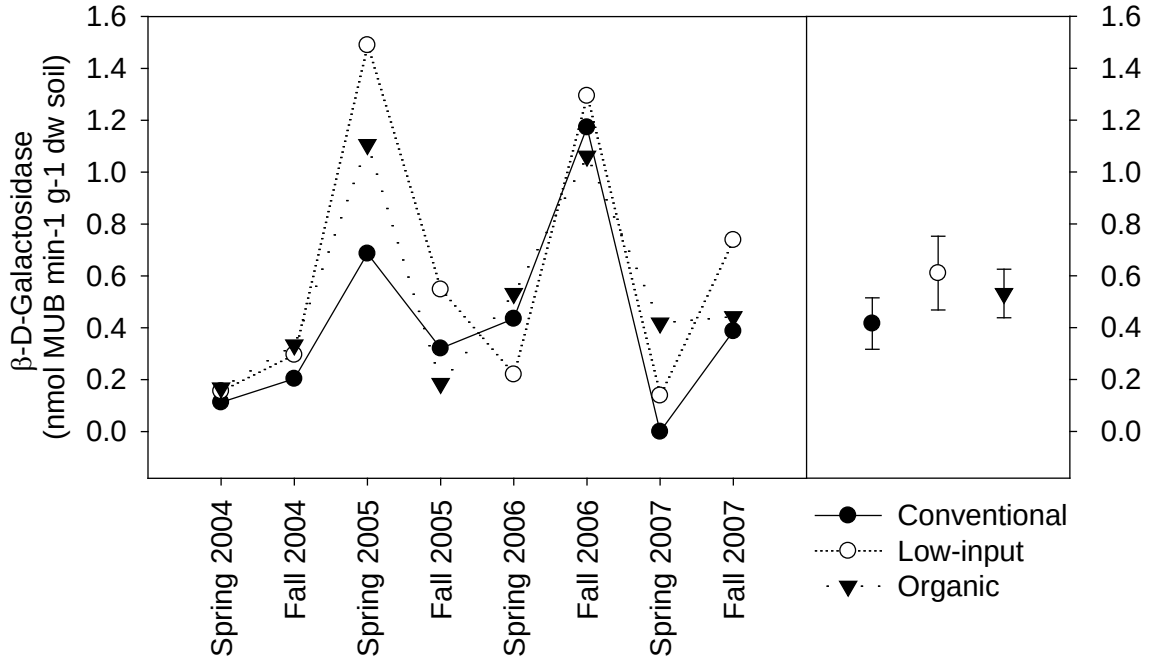


Figure 3.54. β-D-Galactosidase maximum activity under substrate saturation at 0-7 cm soil depth.

Symbols at right indicate the standard error of the grand mean for all sampling times. The presence of different lower-case letters denotes a significant difference using Tukeys HSD (LSMeans). Below are the results of the effect tests in a multi-way ANOVA. An asterisk indicates significance at the level of α = 0.05.

Source	DF	Mean Square	F Ratio	Prob > F
Treatment	2	0.07	0.63	0.5346
Block	3	0.15	1.36	0.2617
Time	1	0.05	0.41	0.5229
Treatment*time	2	0.00	0.01	0.9855
Error	70	0.11		

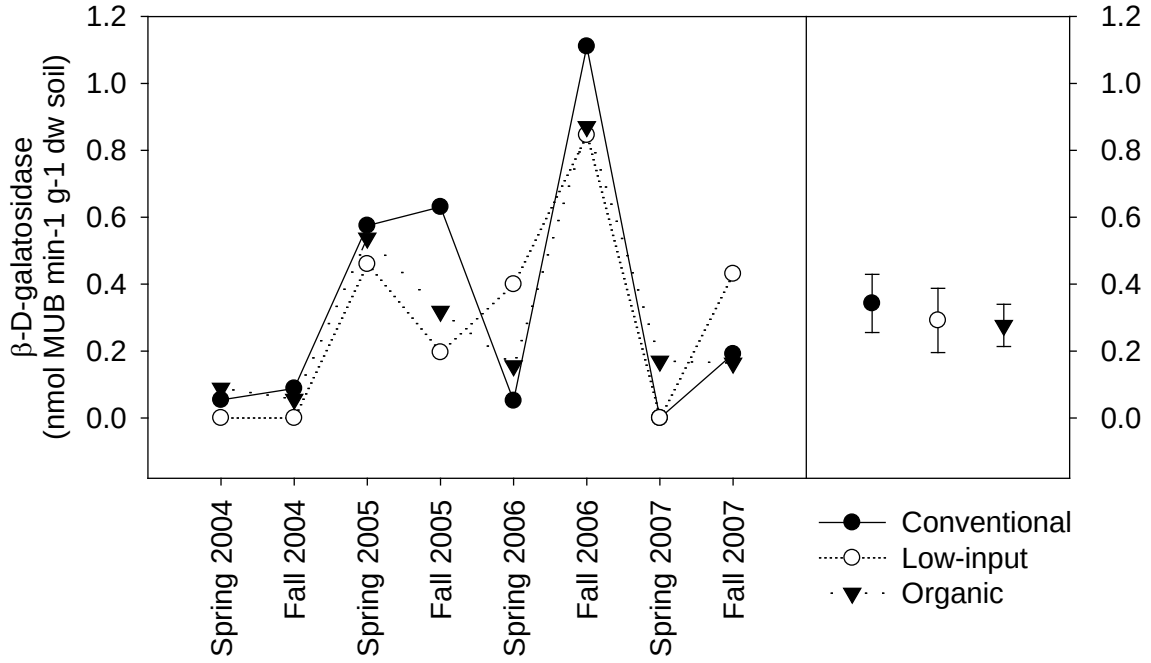


Figure 3.55. β-D-Galactosidase maximum activity under substrate saturation at 7-15 cm soil depth.

Symbols at right indicate the standard error of the grand mean for all sampling times. The presence of different lower-case letters denotes a significant difference using Tukeys HSD (LSMeans). Below are the results of the effect tests in a multi-way ANOVA. An asterisk indicates significance at the level of α = 0.05.

Source	DF	Mean Square	F Ratio	Prob > F
Treatment	2	0.01	0.14	0.8682
Block	3	0.01	0.14	0.9345
Time	1	0.07	0.85	0.3610
Treatment*time	2	0.02	0.29	0.7474
Error	69	0.08		

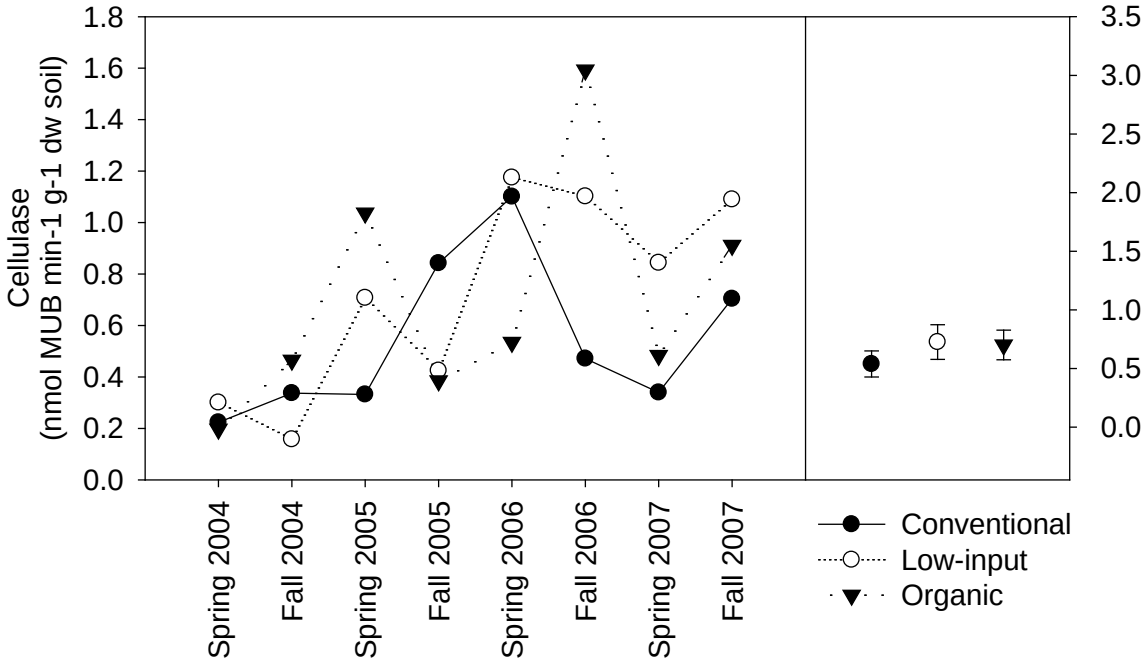


Figure 3.56. Cellulase maximum activity under substrate saturation at 0-7 cm soil depth.

Symbols at right indicate the standard error of the grand mean for all sampling times. The presence of different lower-case letters denotes a significant difference using Tukeys HSD (LSMeans). Below are the results of the effect tests in a multi-way ANOVA. An asterisk indicates significance at the level of α = 0.05.

Source	DF	Mean Square	F Ratio	Prob > F
Treatment	2	0.08	0.81	0.4498
Block	3	0.68	6.66	*0.0005
Time	1	0.97	9.48	*0.0030
Treatment*time	2	0.05	0.48	0.6204
Error	70	0.10		

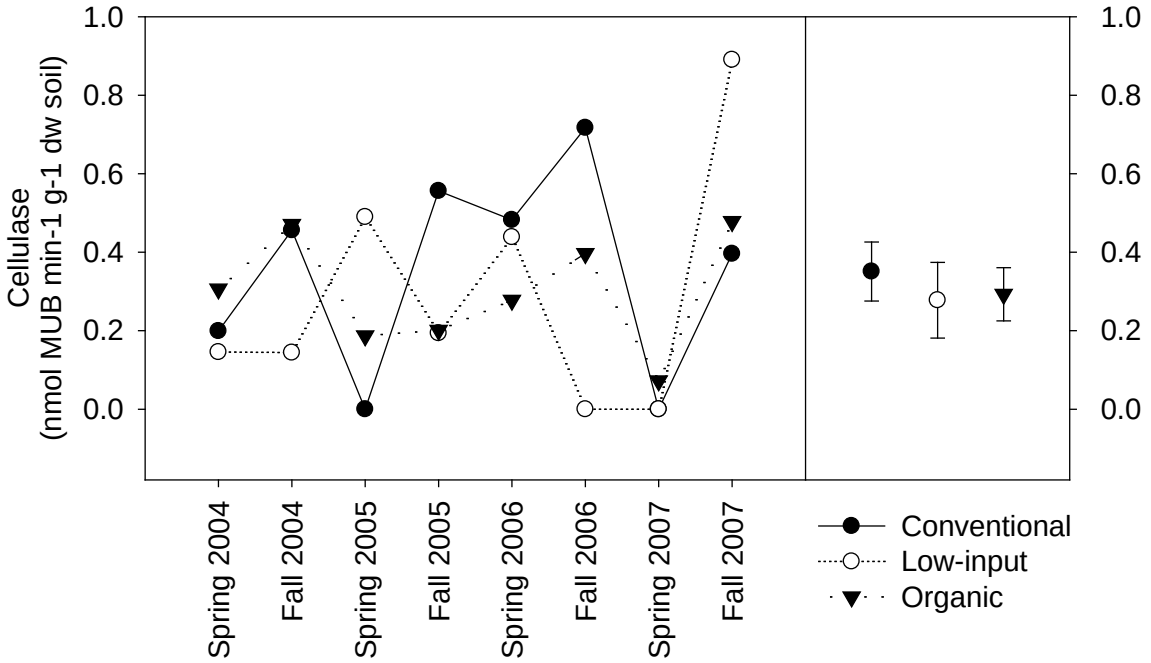


Figure 3.57. Cellulase maximum activity under substrate saturation at 7-15 cm soil depth.

Symbols at right indicate the standard error of the grand mean for all sampling times. The presence of different lower-case letters denotes a significant difference using Tukeys HSD (LSMeans). Below are the results of the effect tests in a multi-way ANOVA. An asterisk indicates significance at the level of α = 0.05.

Source	DF	Mean Square	F Ratio	Prob > F
Treatment	2	0.01	0.04	0.9576
Block	3	0.38	3.21	*0.0281
Time	1	0.15	1.29	0.2604
Treatment*time	2	0.05	0.40	0.6746
Error	71	0.12		

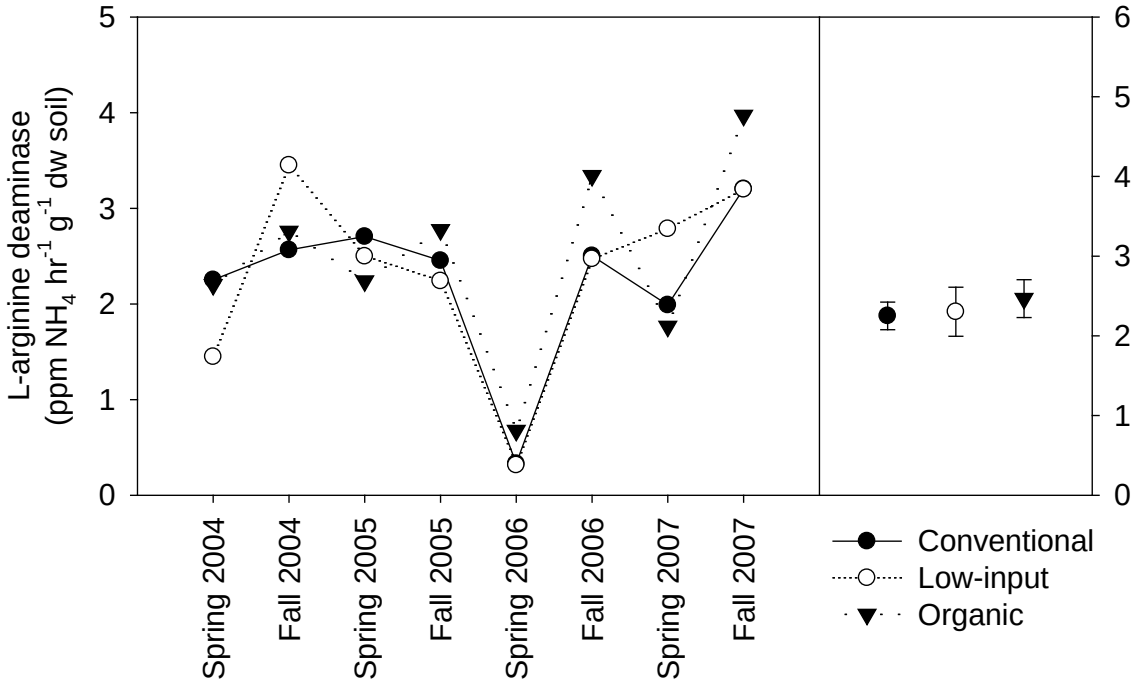


Figure 3.58. L-arginine deaminase maximum activity under substrate saturation at 0-7 cm soil depth.

Symbols at right indicate the standard error of the grand mean for all sampling times. The presence of different lower-case letters denotes a significant difference using Tukeys HSD (LSMeans). Below are the results of the effect tests in a multi-way ANOVA. An asterisk indicates significance at the level of α = 0.05.

Source	DF	Mean Square	F Ratio	Prob > F
Treatment	2	0.67	0.48	0.6235
Block	3	2.82	2.02	0.1195
Time	1	0.05	0.04	0.8471
Treatment*time	2	0.19	0.14	0.8729
Error	71	1.40		

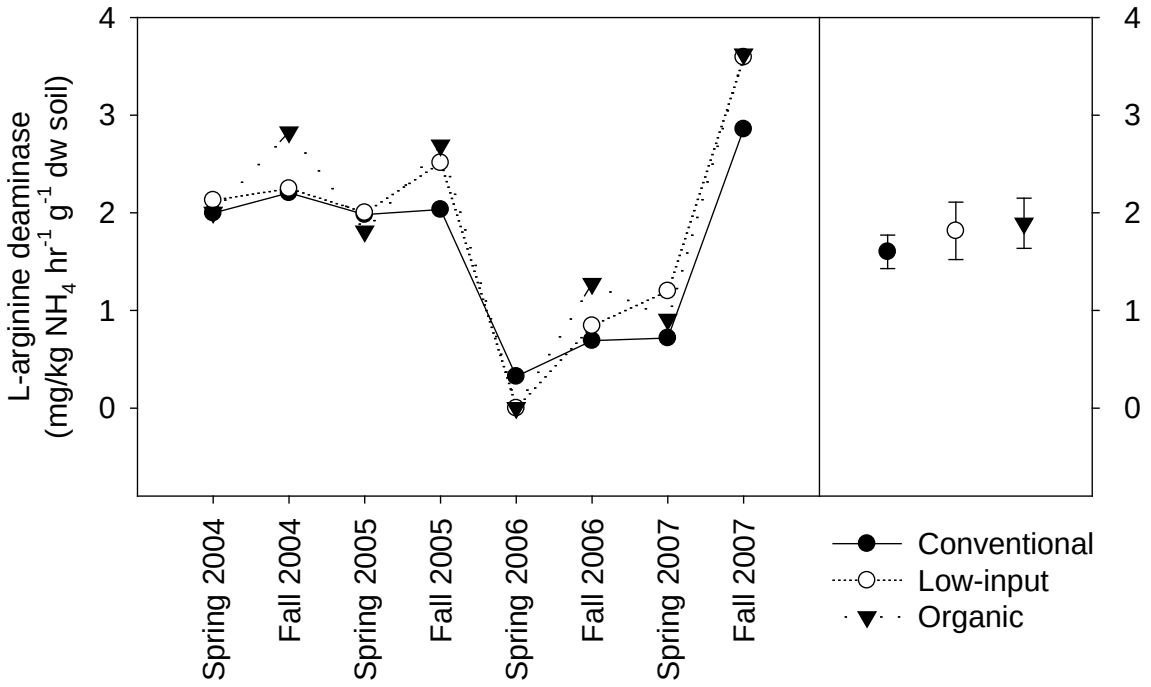


Figure 3.59. L-arginine deaminase maximum activity under substrate saturation at 7-15 cm soil depth.

Symbols at right indicate the standard error of the grand mean for all sampling times. The presence of different lower-case letters denotes a significant difference using Tukeys HSD (LSMeans). Below are the results of the effect tests in a multi-way ANOVA. An asterisk indicates significance at the level of α = 0.05.

Source	DF	Mean Square	F Ratio	Prob > F
Treatment	2	0.71	0.48	0.6188
Block	3	2.15	1.45	0.2348
Time	1	3.26	2.21	0.1419
Treatment*time	2	0.10	0.07	0.9338
Error	71	1.48		

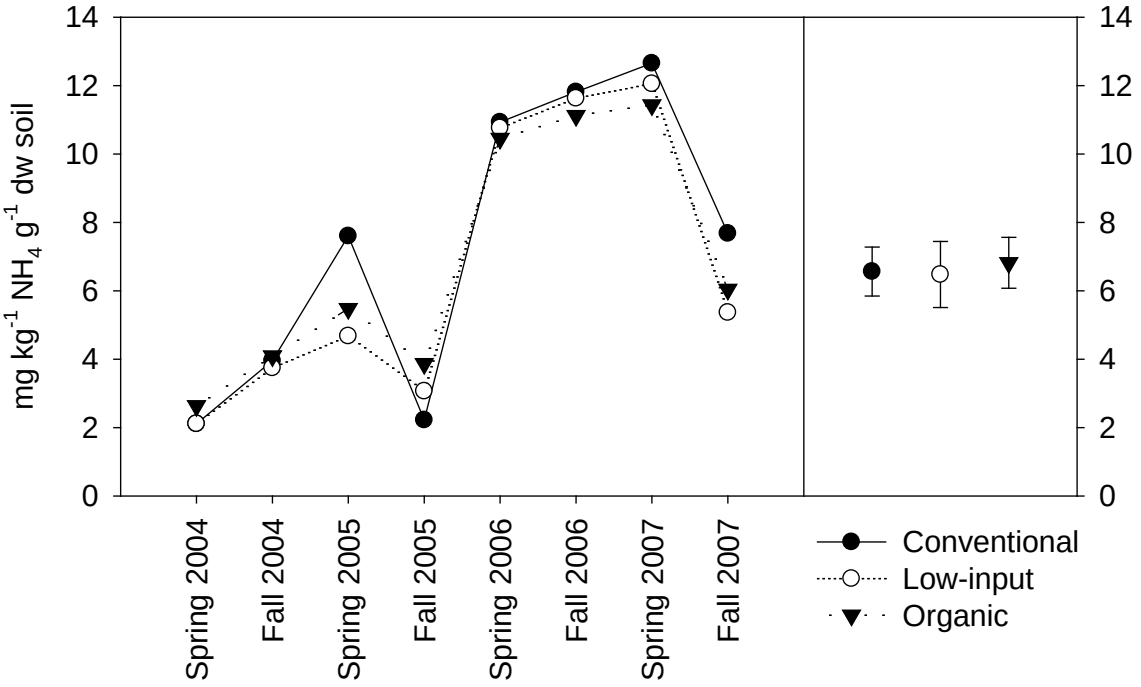


Figure 3.60. Mineral nitrogen (as NH_4) at 0-7 cm.
Symbols at right indicate the standard error of the grand mean for all sampling times. The presence of different lower-case letters denotes a significant difference using Tukeys HSD (LSMeans). Below are the results of the effect tests in a multi-way ANOVA. An asterisk indicates significance at the level of α = 0.05.

Source	DF	Mean Square	F Ratio	Prob > F
Treatment	2	0.39	0.03	0.9659
Block	3	5.71	0.51	0.6749
Time	1	500.67	44.91	*<.0001
Treatment*Time	2	0.07	0.01	0.9934
Error	71	11.15		

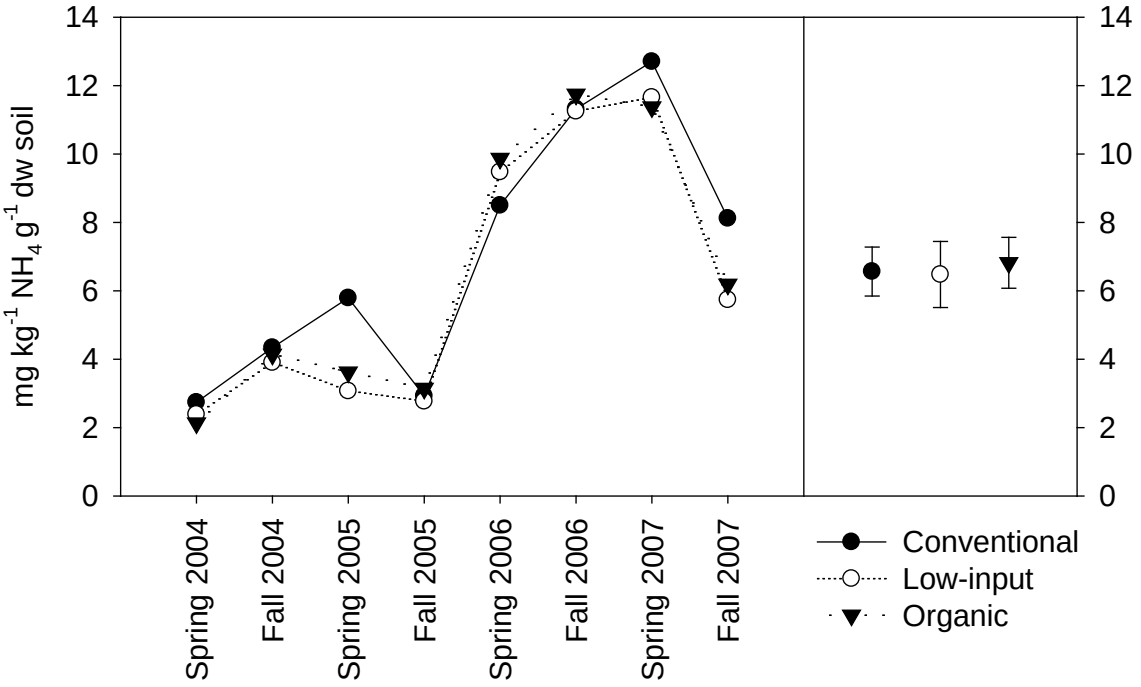


Figure 3.61. Mineral nitrogen (as NH_4) at 7-15 cm.
Symbols at right indicate the standard error of the grand mean for all sampling times. The presence of different lower-case letters denotes a significant difference using Tukeys HSD (LSMeans). Below are the results of the effect tests in a multi-way ANOVA. An asterisk indicates significance at the level of α = 0.05.

Source	DF	Mean Square	F Ratio	Prob > F
Treatment	2	0.65	0.07	0.9349
Block	3	6.74	0.70	0.5552
Time	1	504.72	52.41	*<.0001
Treatment*Time	2	3.91	0.41	0.668
Error	71	9.63		

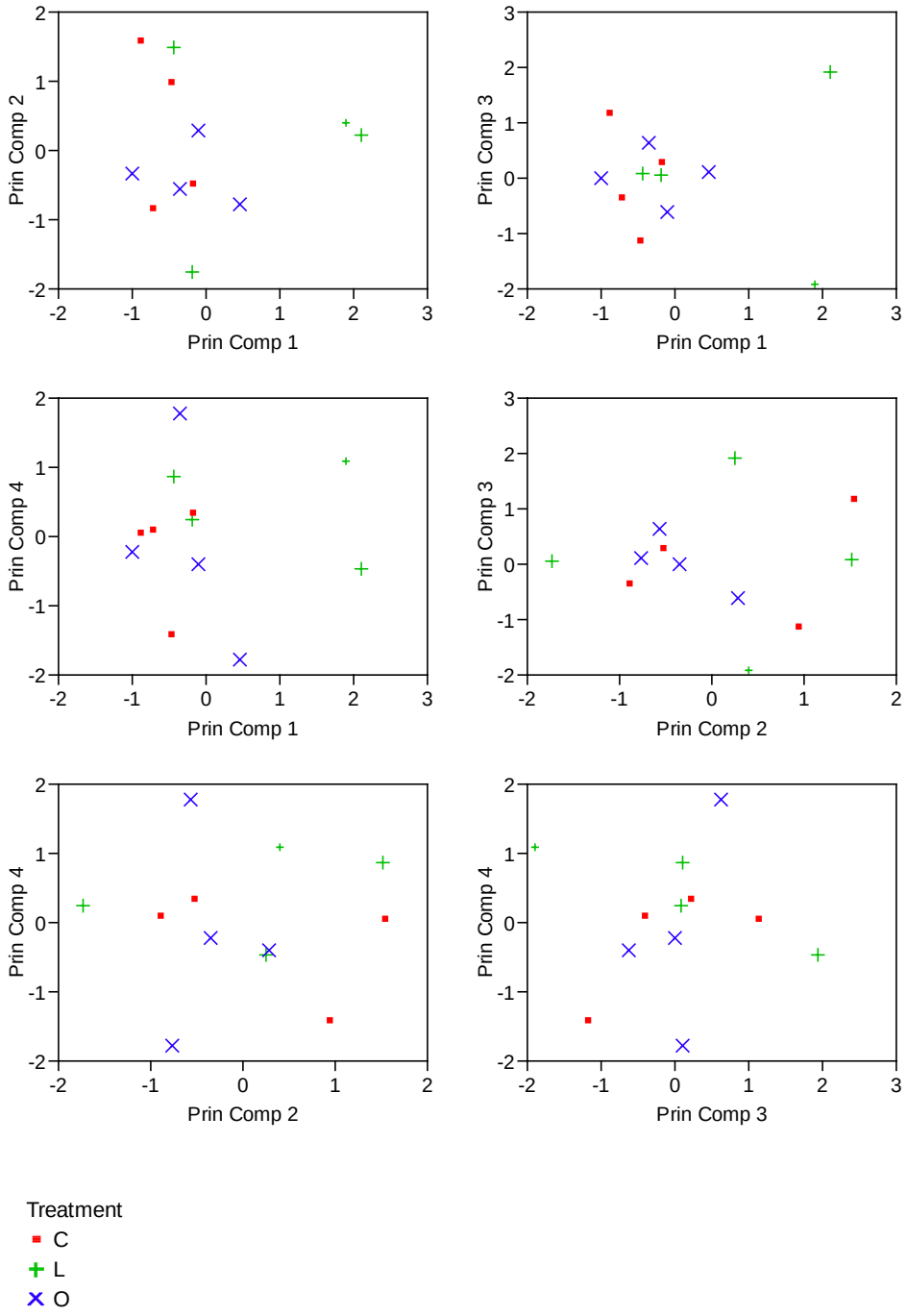


Figure 3.62. PCA overlay plots for Spring 2004, 0-7 cm

PC 1-4 represents 75.468% of the total variability in the data set.

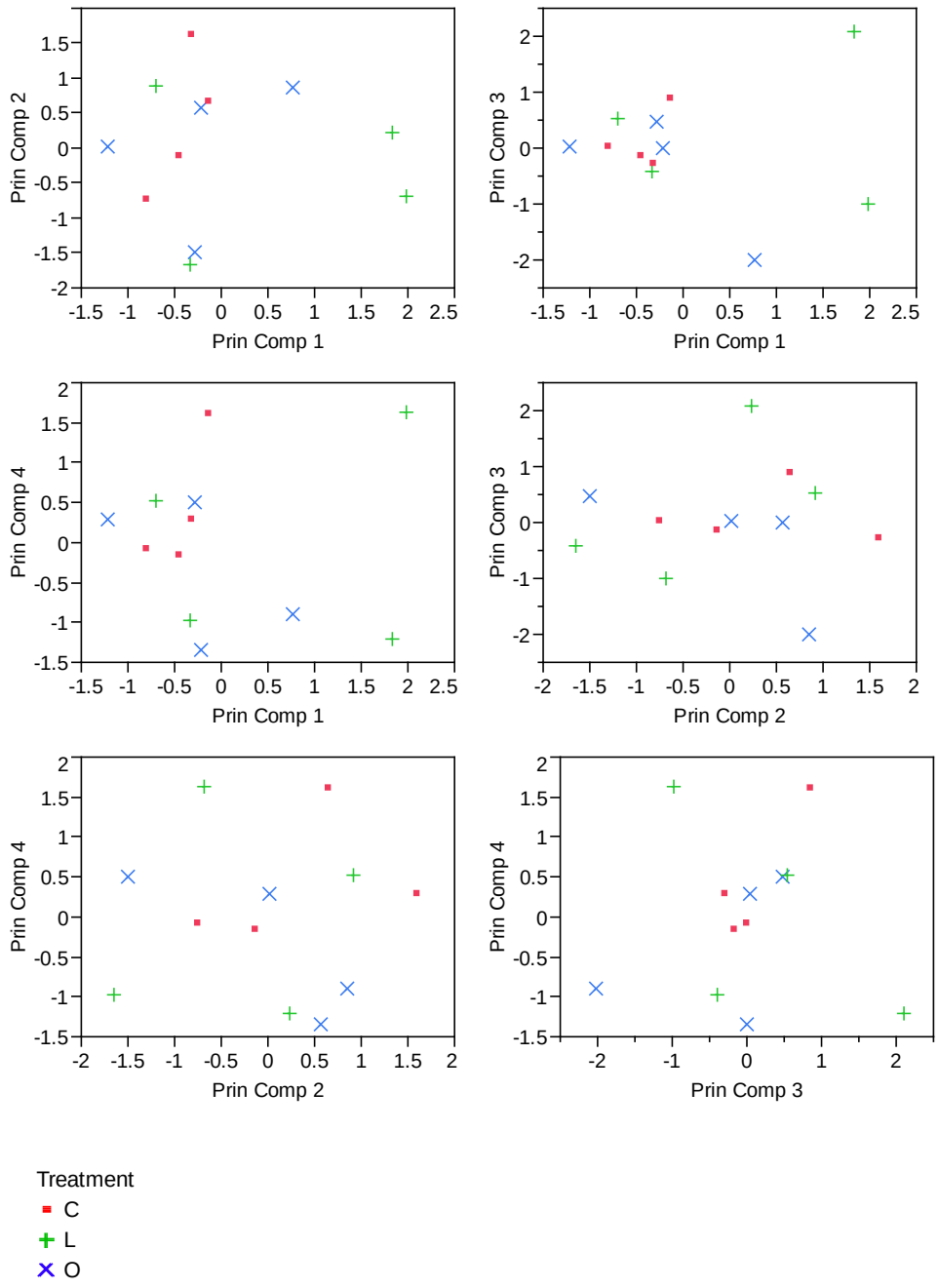


Figure 3.63. PCA overlay plots for Spring 2004, 7-15 cm

PC 1-4 represents 82.181% of the total variability in the data set.

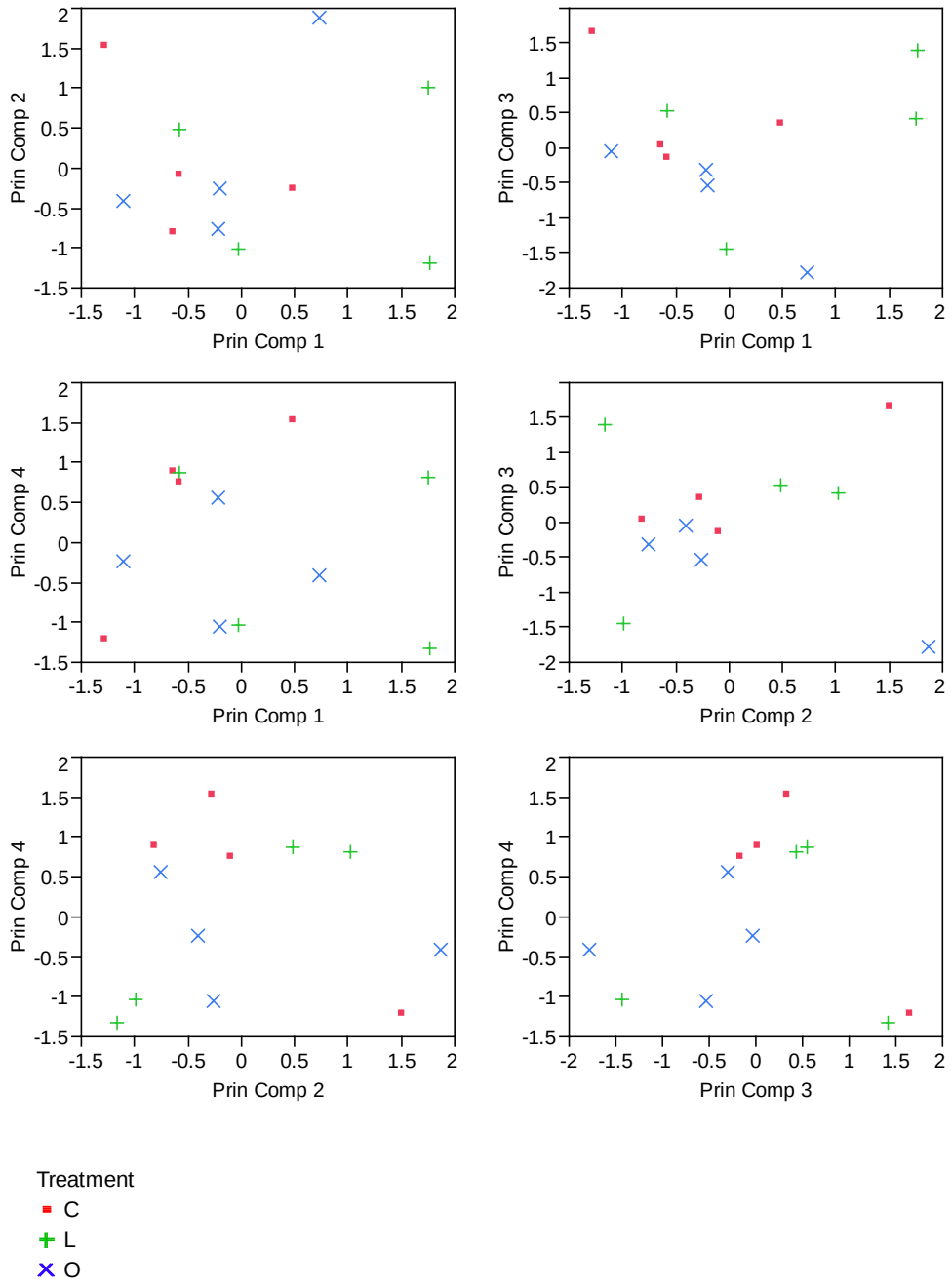


Figure 3.64. PCA overlay plots for Fall 2004, 0-7 cm

PC 1-4 represents 82.741% of the total variability in the data set.

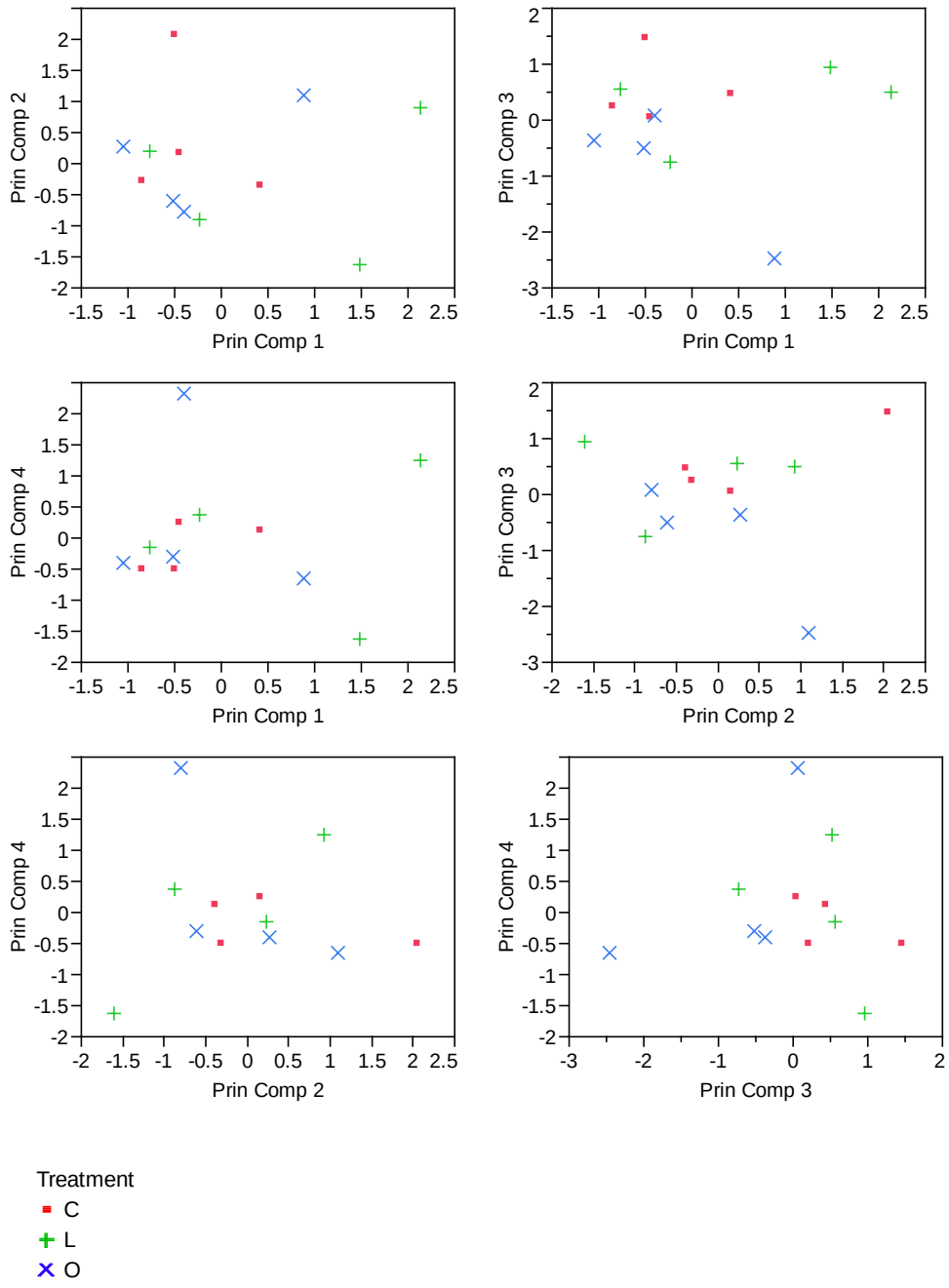


Figure 3.65. PCA overlay plots for Fall 2004, 7-15 cm

PC 1-4 represents 84.296% of the total variability in the data set.

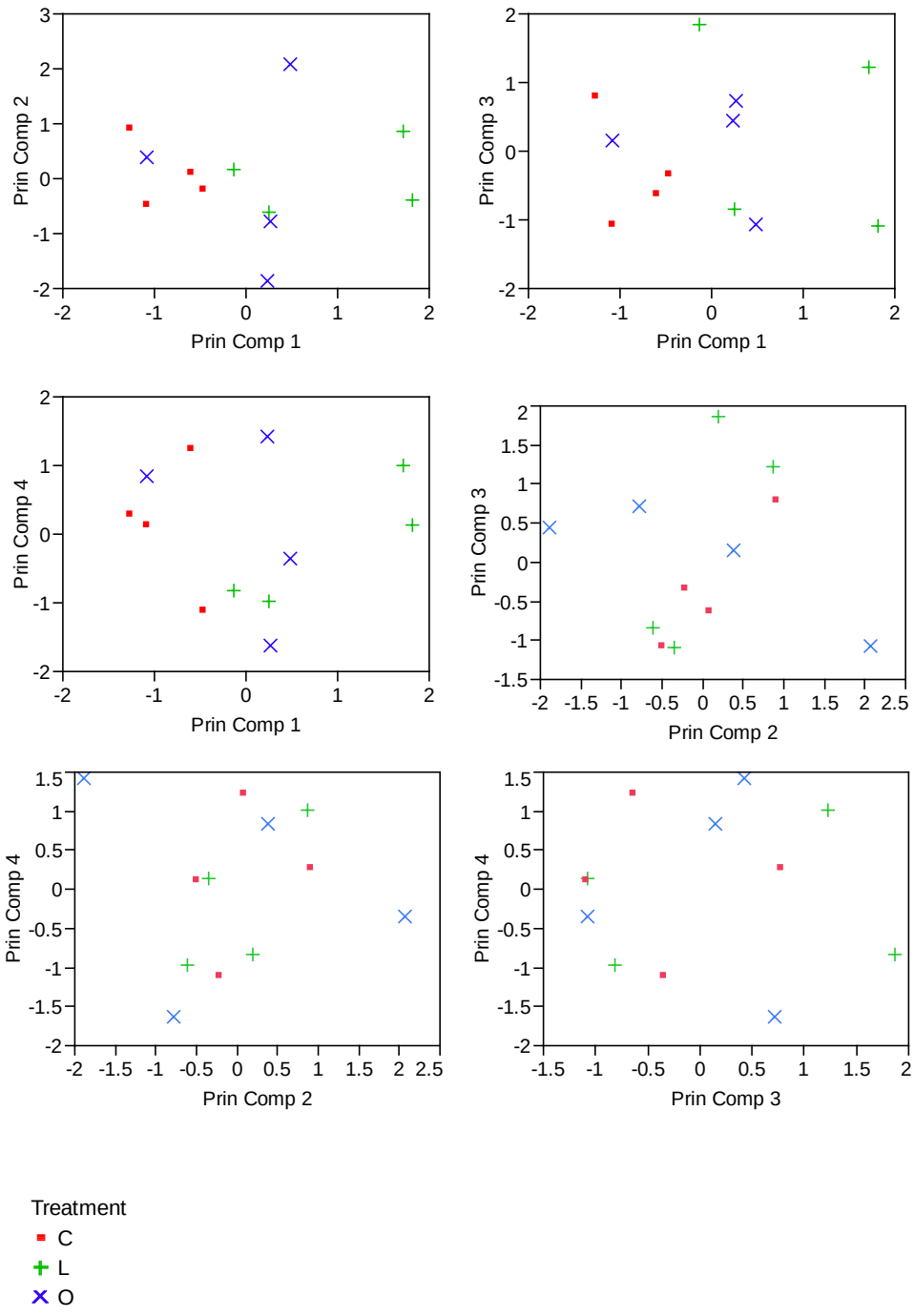


Figure 3.66. PCA overlay plots for Spring 2005, 0-7 cm

PC 1-4 represents 78.593% of the total variability in the data set.

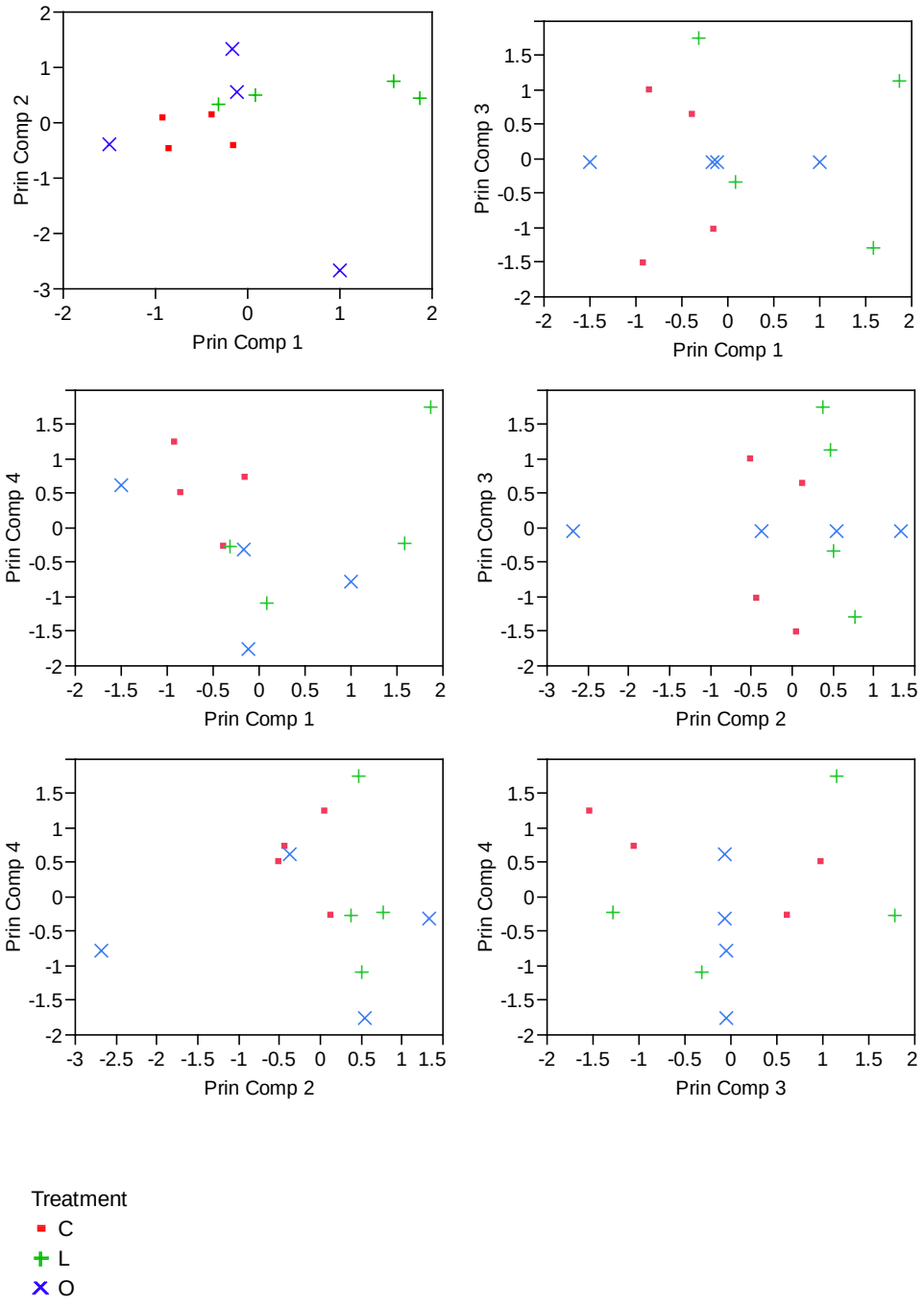


Figure 3.67. PCA overlay plots for Spring 2005, 7-15 cm

PC 1-4 represents 81.087% of the total variability in the data set.

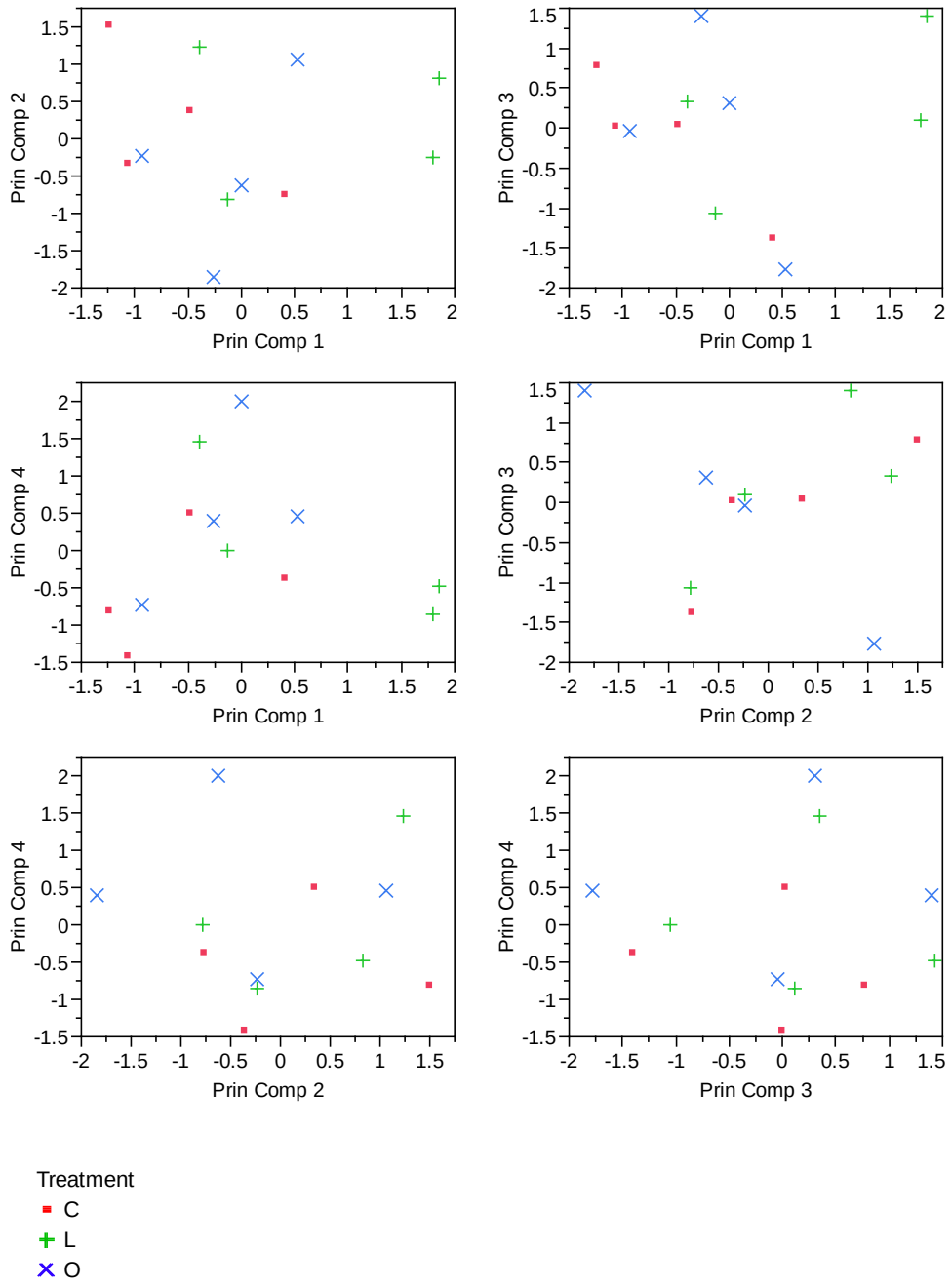


Figure 3.68. PCA overlay plots for Fall 2005, 0-7 cm

PC 1-4 represents 77.282% of the total variability in the data set.

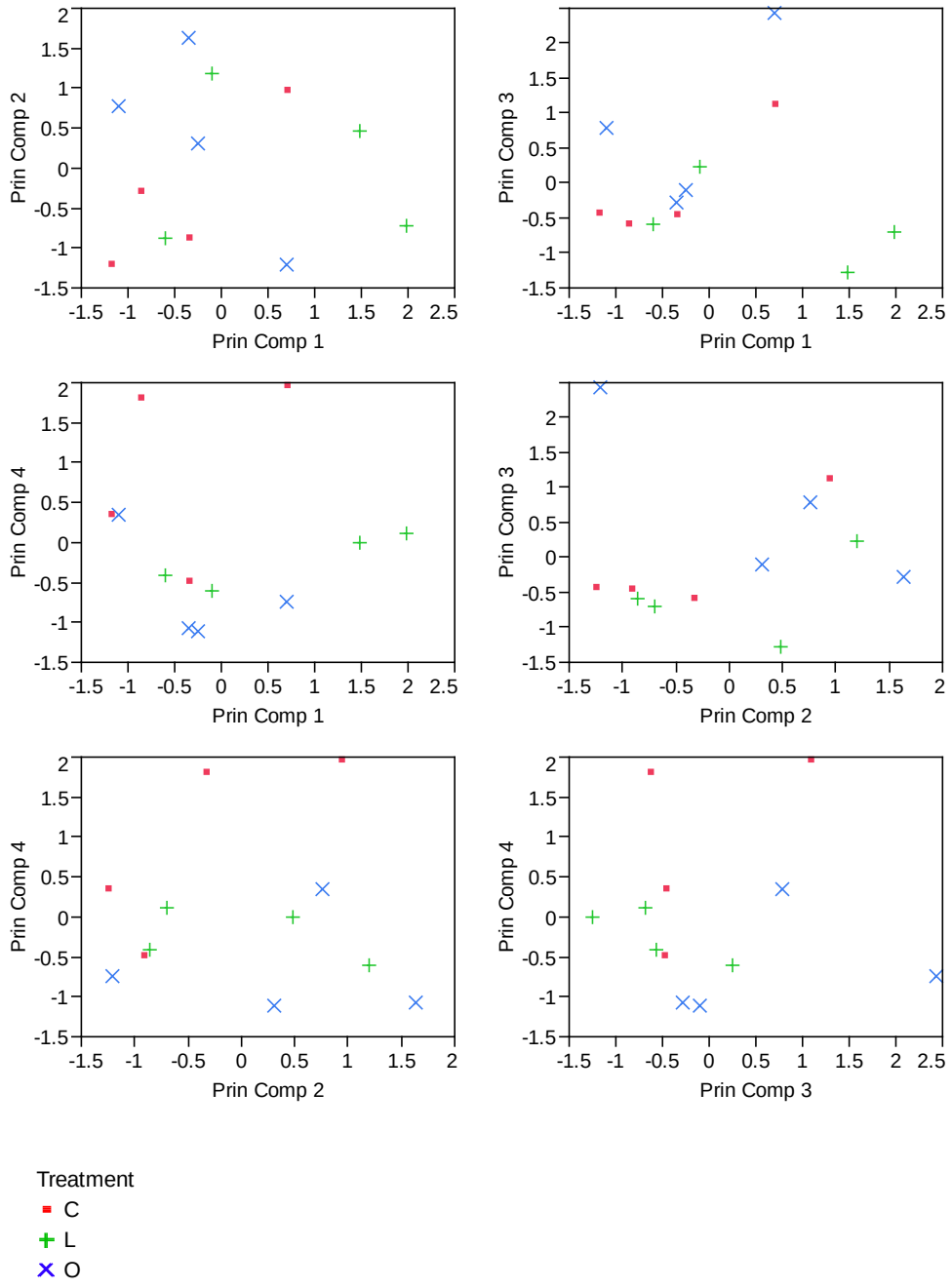


Figure 3.69. PCA overlay plots for Fall 2005, 7-15 cm

PC 1-4 represents 80.620% of the total variability in the data set.

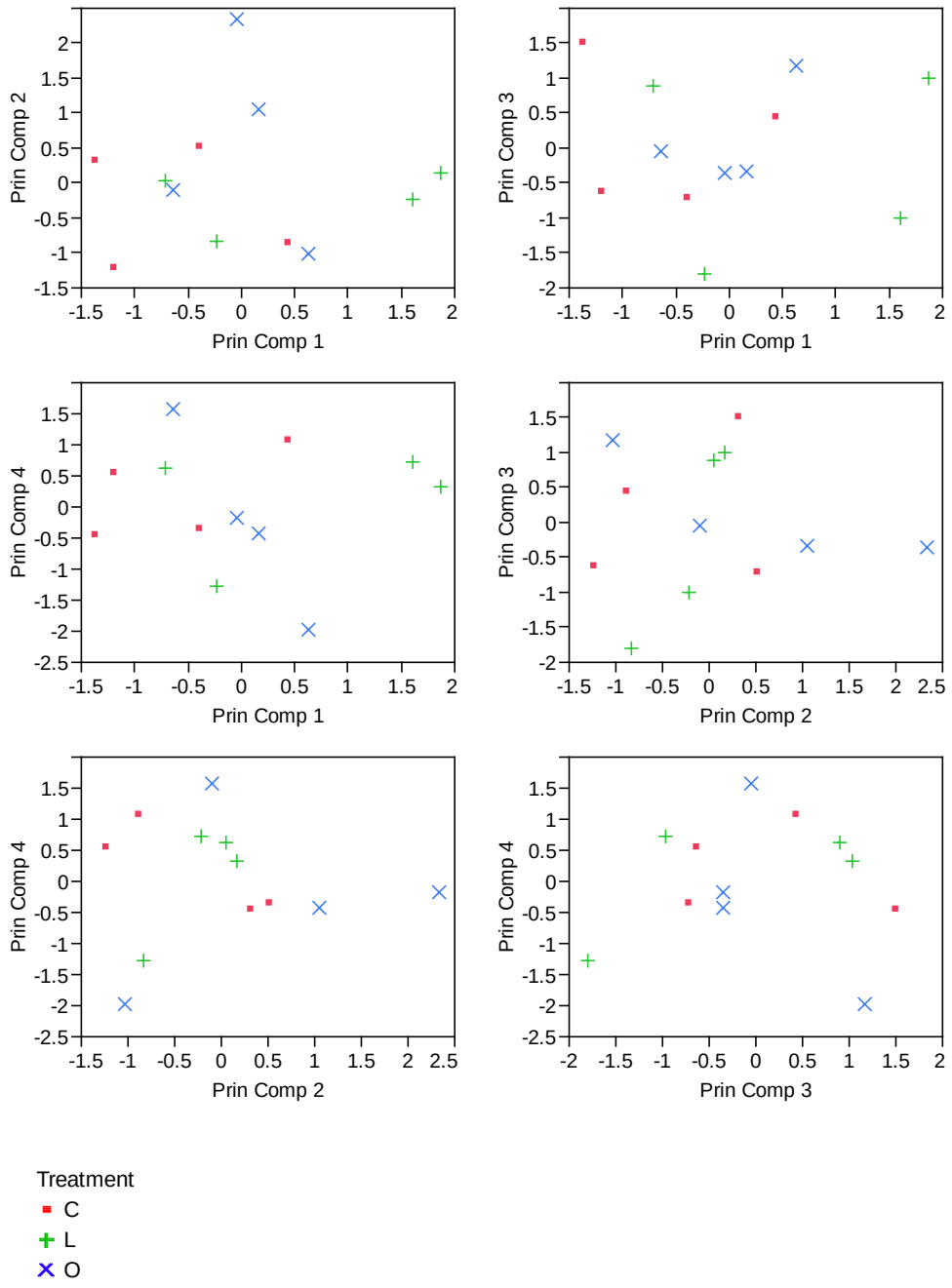


Figure 3.70. PCA overlay plots for Spring 2006, 0-7 cm

PC 1-4 represent 80.65% of total variability in the data set

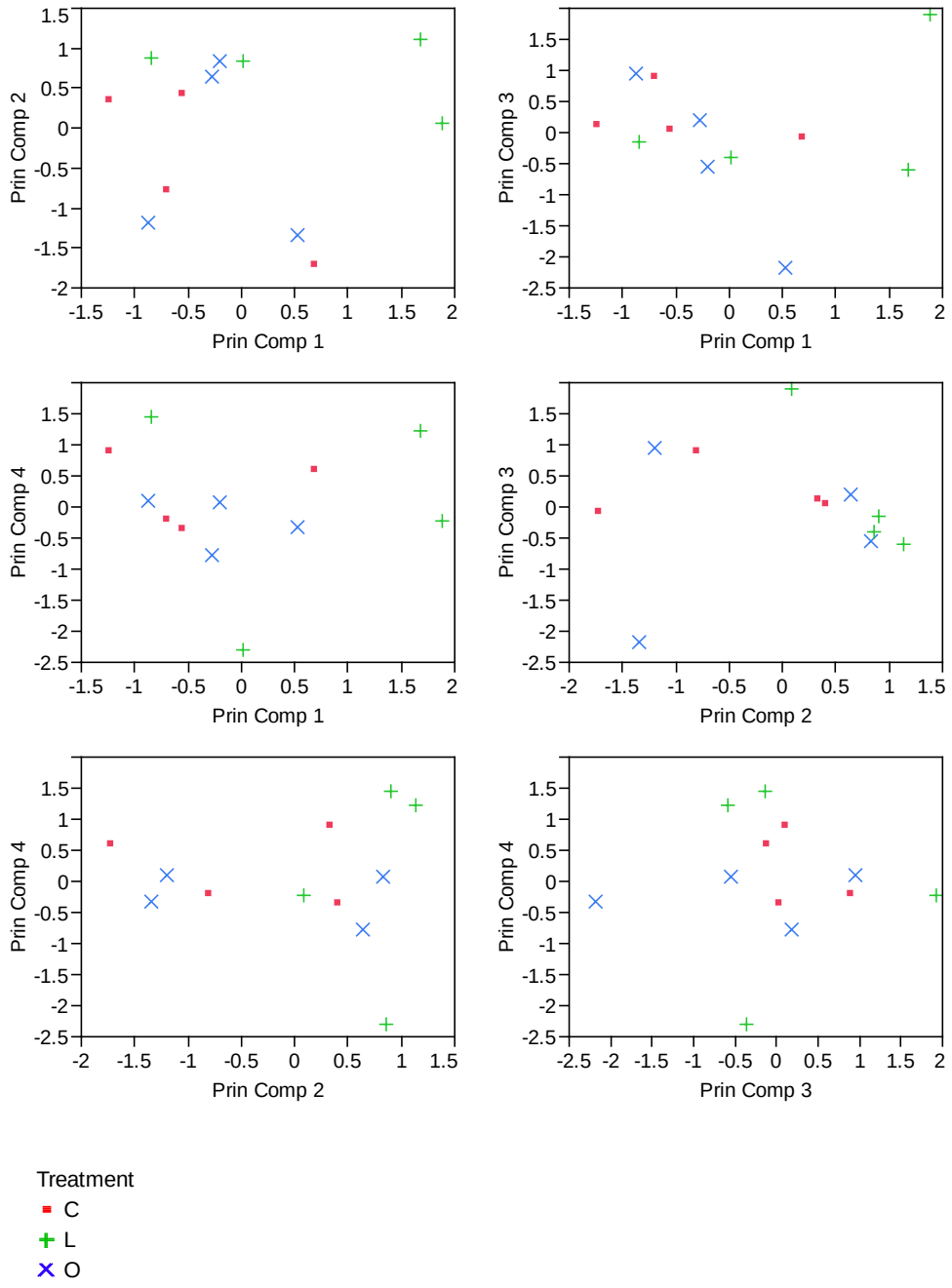


Figure 3.71. PCA overlay plots for Spring 2006, 7-15 cm

PC 1-4 represent 81.324% of the total variability in the data set

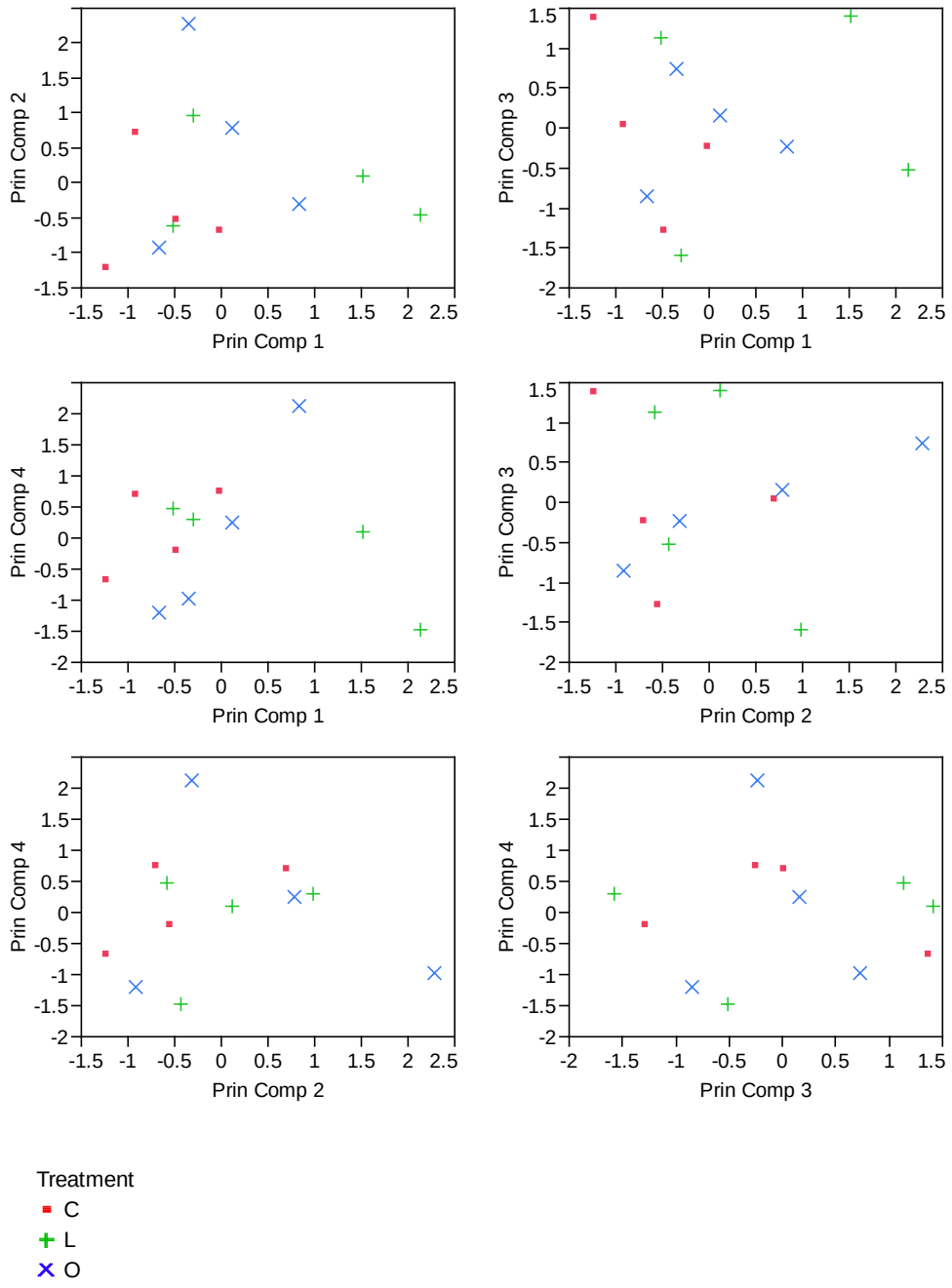


Figure 3.72. PCA overlay plots for Fall 2006, 0-7 cm

PC 1-4 represents 84.482% of the total variability in the data set.

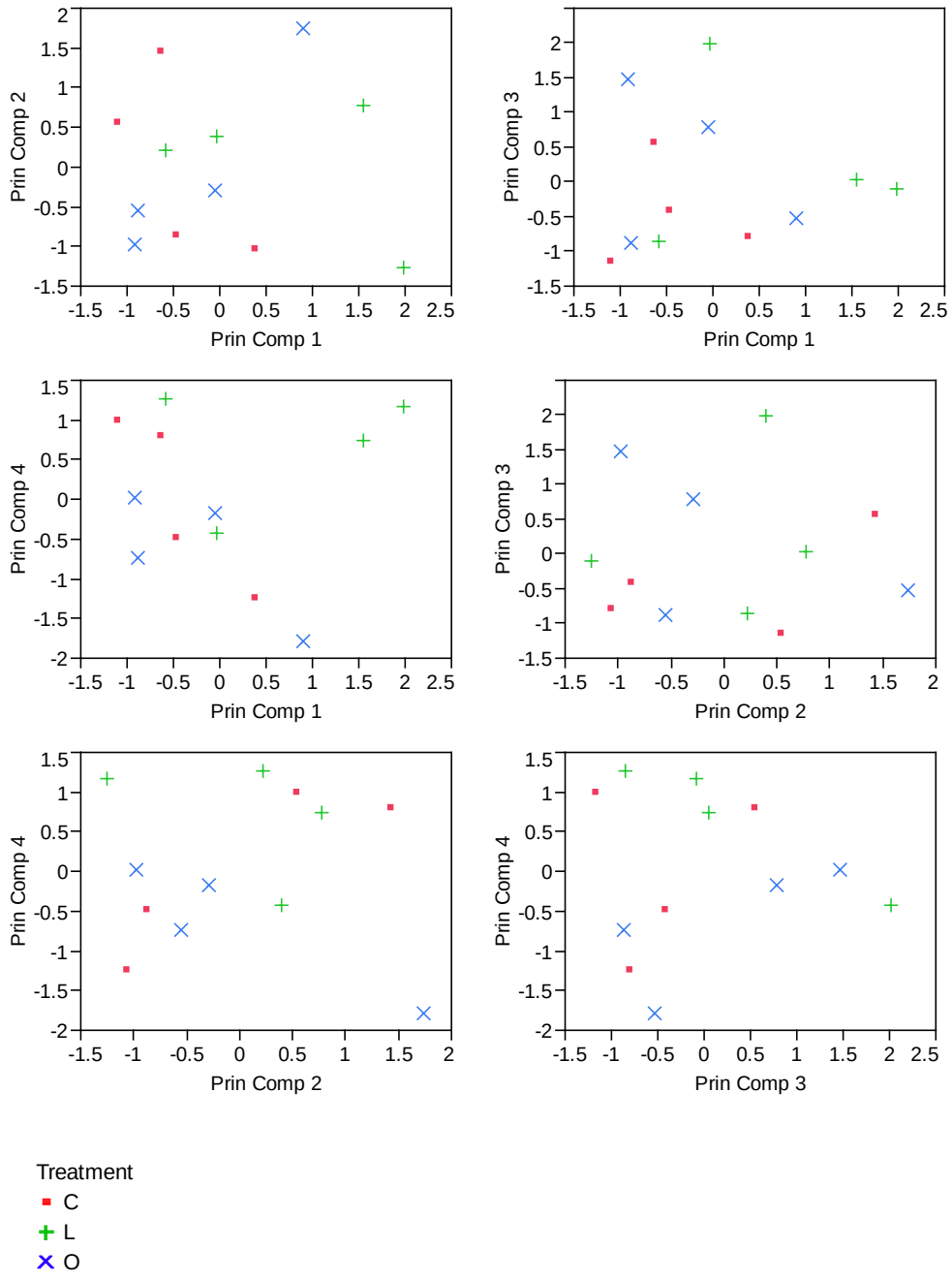


Figure 3.73. PCA overlay plots for Fall 2006, 7-15 cm

PC 1-4 represents 79.068% of the total variability in the data set.

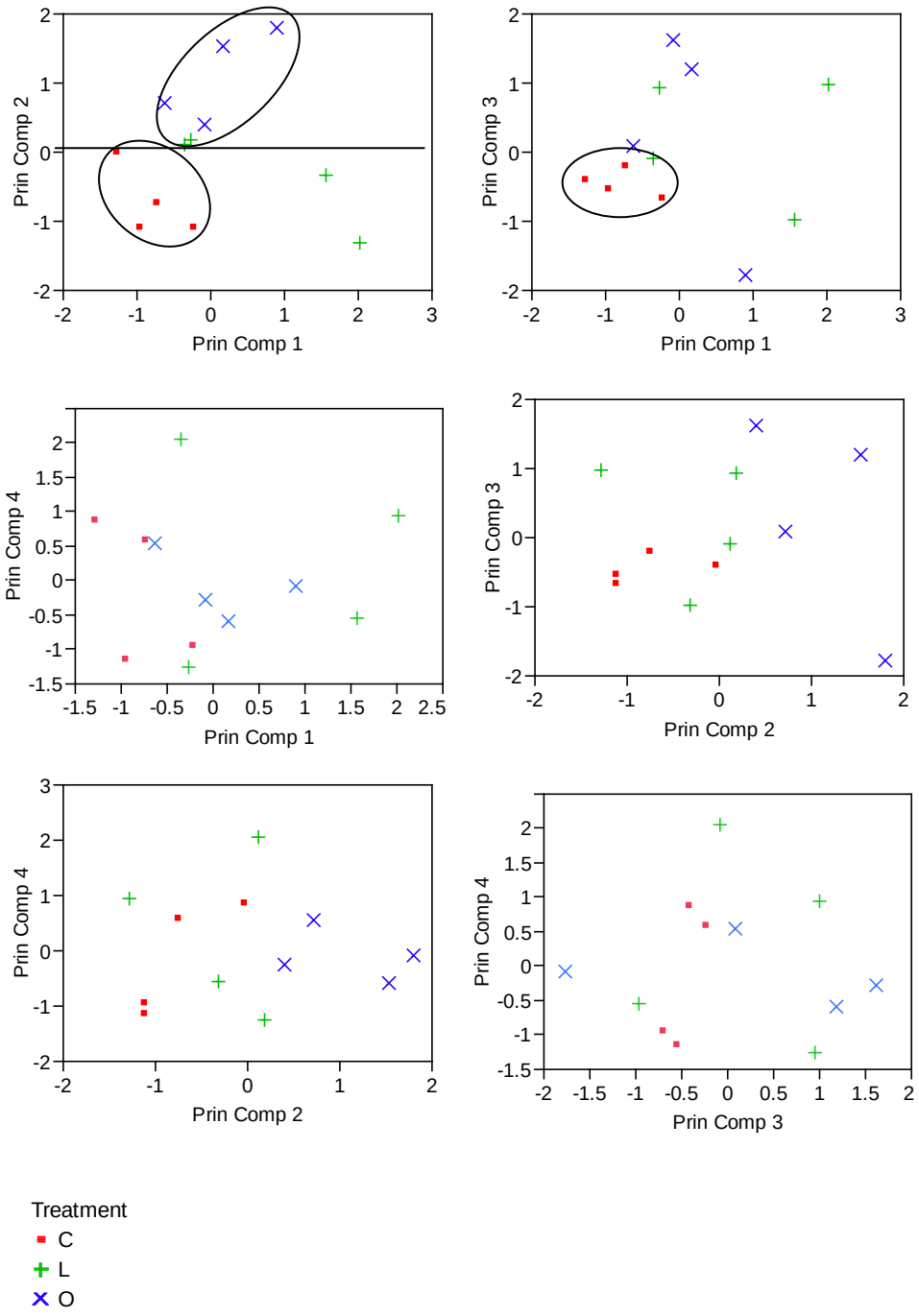


Figure 3.74. PCA overlay plots for Spring 2007, 0-7 cm.

PC 1-4 represents 80.482% of the total variability in the data set. Treatments grouped by PCA are circled.

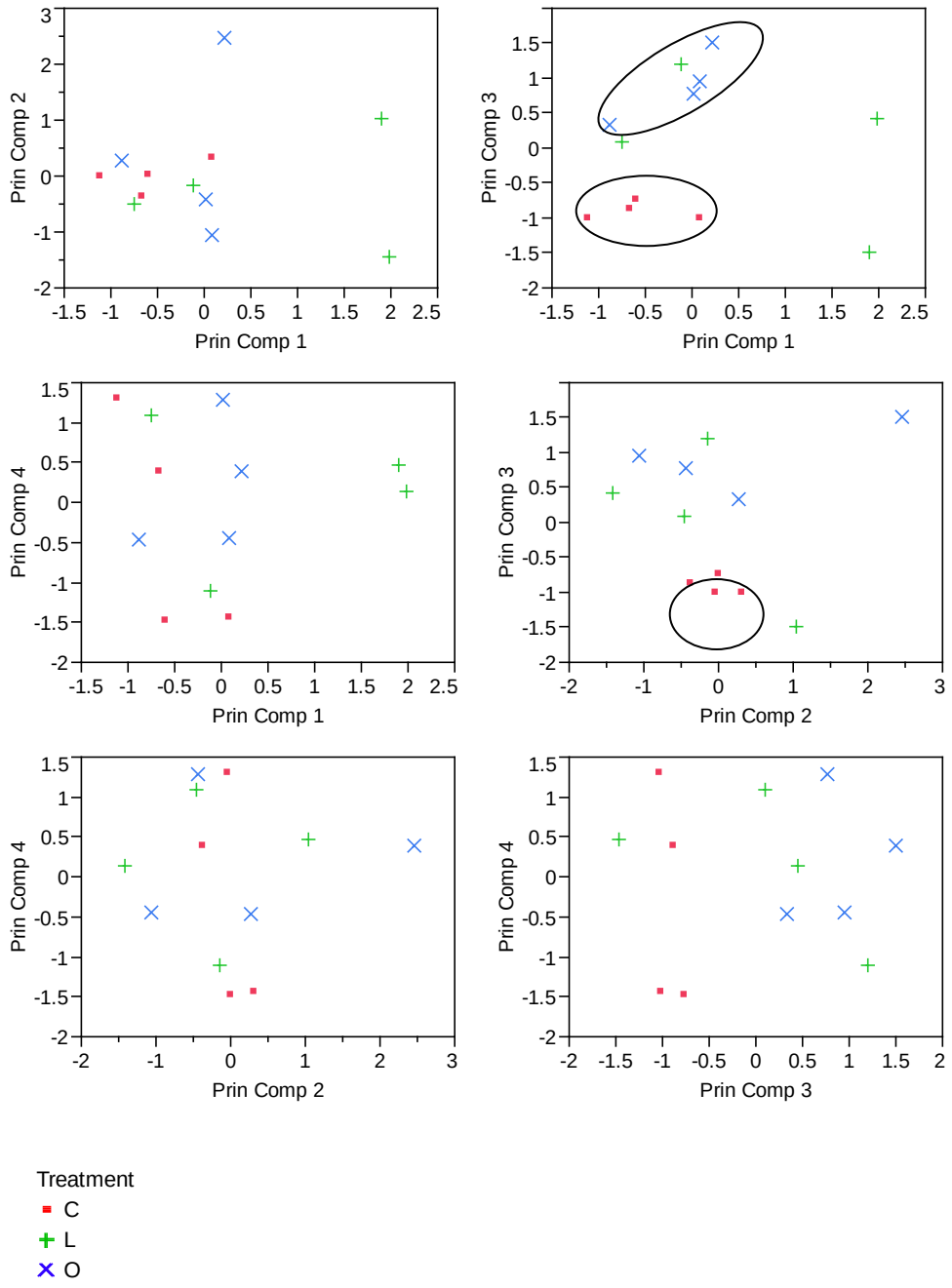


Figure 3.75. PCA overlay plots for Spring 2007, 7-15 cm

PC 1-4 represent 80.984% of the total variability in the data set. Treatments grouped by PCA are circled.

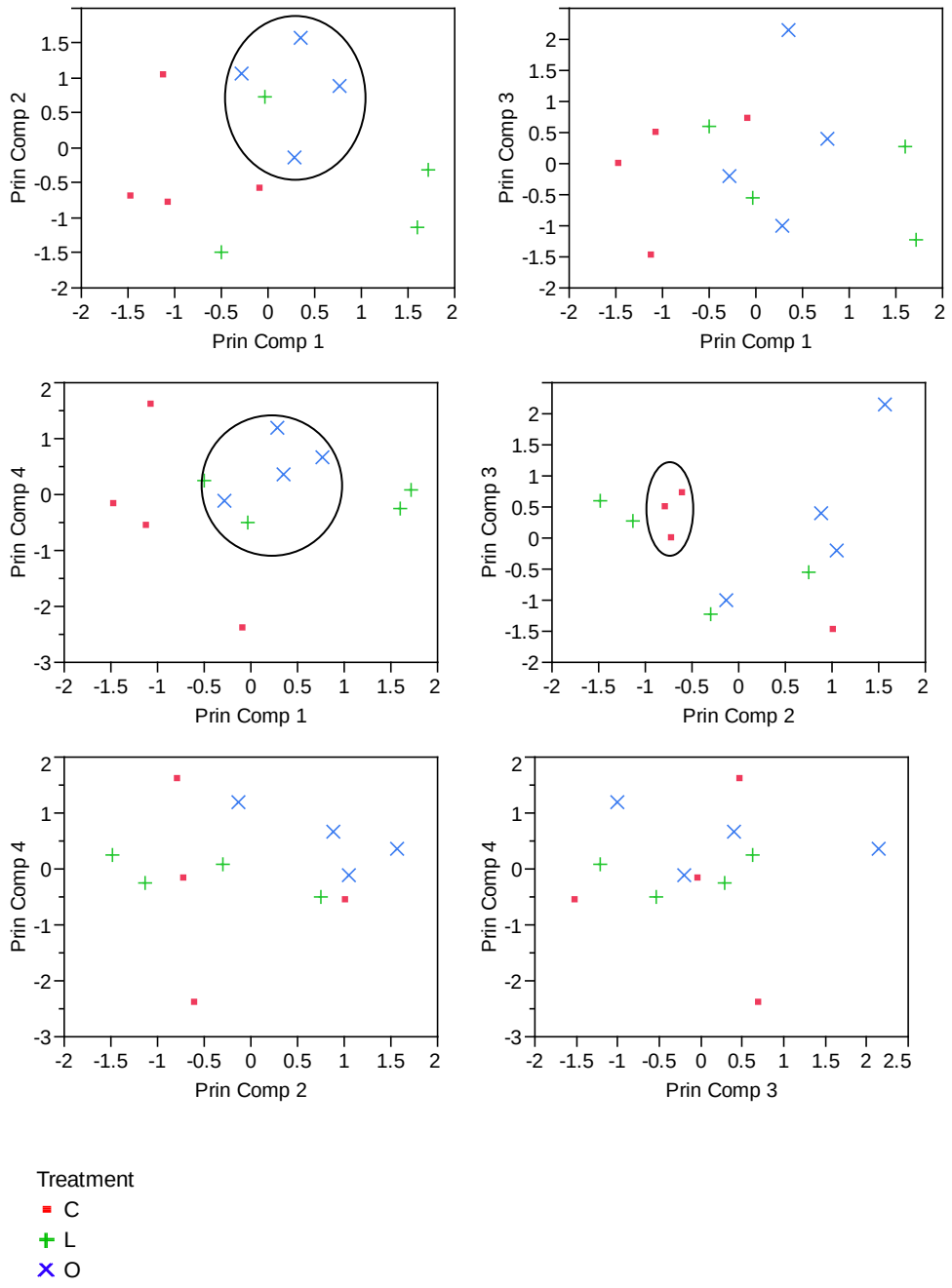


Figure 3.76. PCA overlay plots for Fall 2007, 0-7 cm

PC 1-4 represents 78.580% of the total variability in the data set. Treatments grouped by PCA are circled.

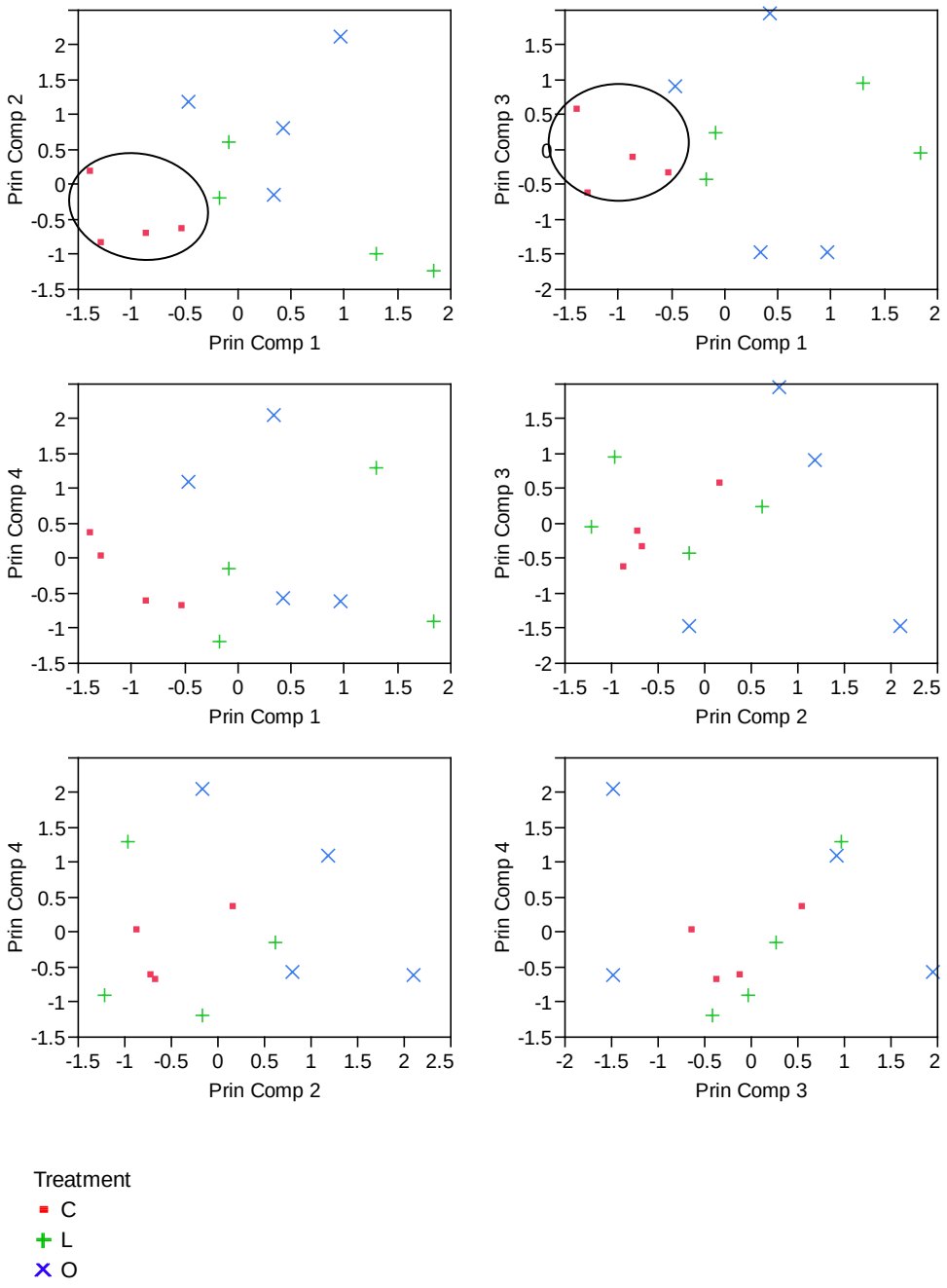


Figure 3.77. PCA overlay plots for Fall 2007, 7-15 cm

PC 1-4 represents 79.422% of the total variability in the data set.

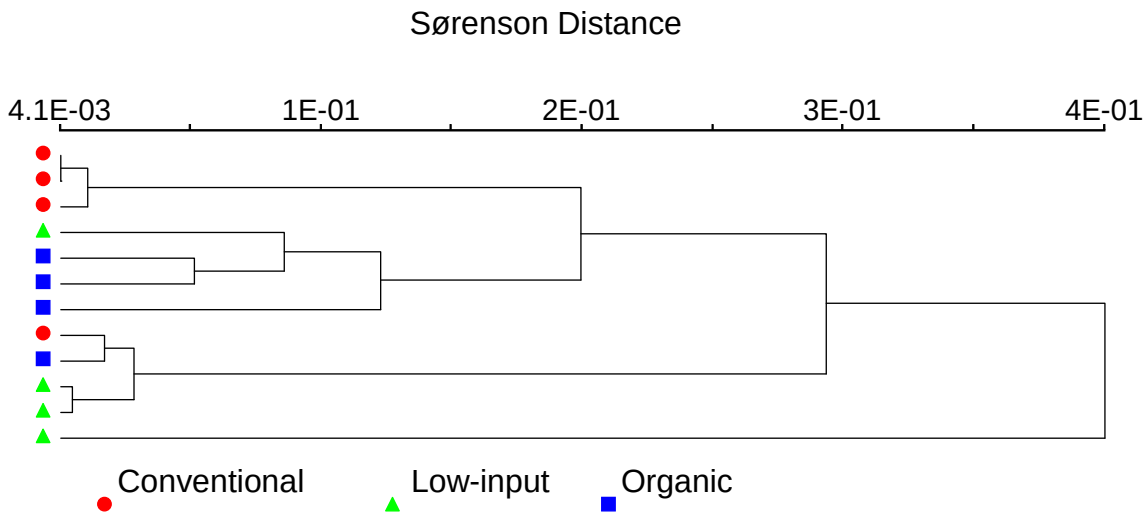


Figure 3.78**.** Hierarchical clustering of bacterial T-RFLP profiles in 2007.

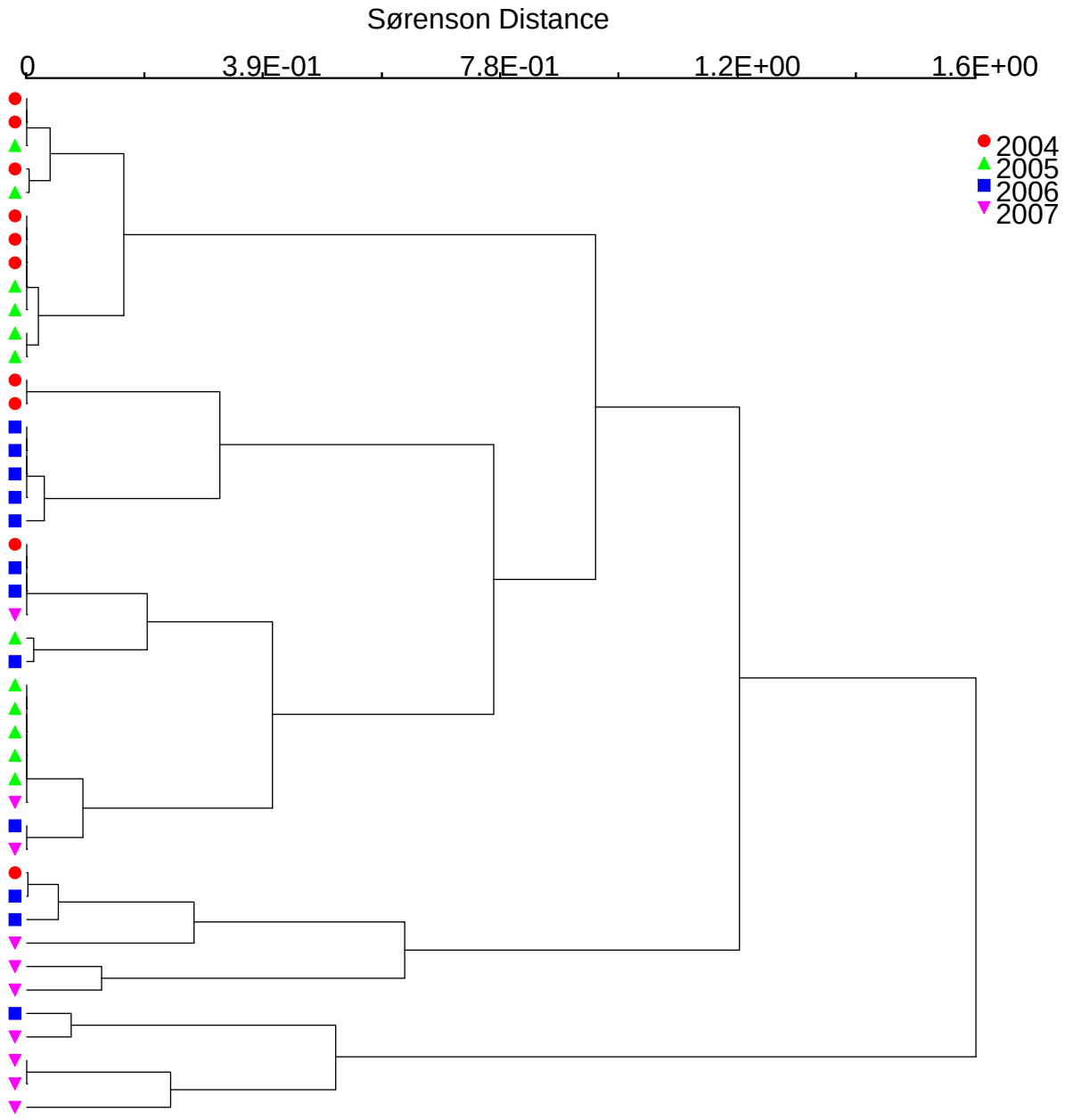


Figure 3.79. Hierarchical clustering dendrogram of fungal profiles from years 2004-2007

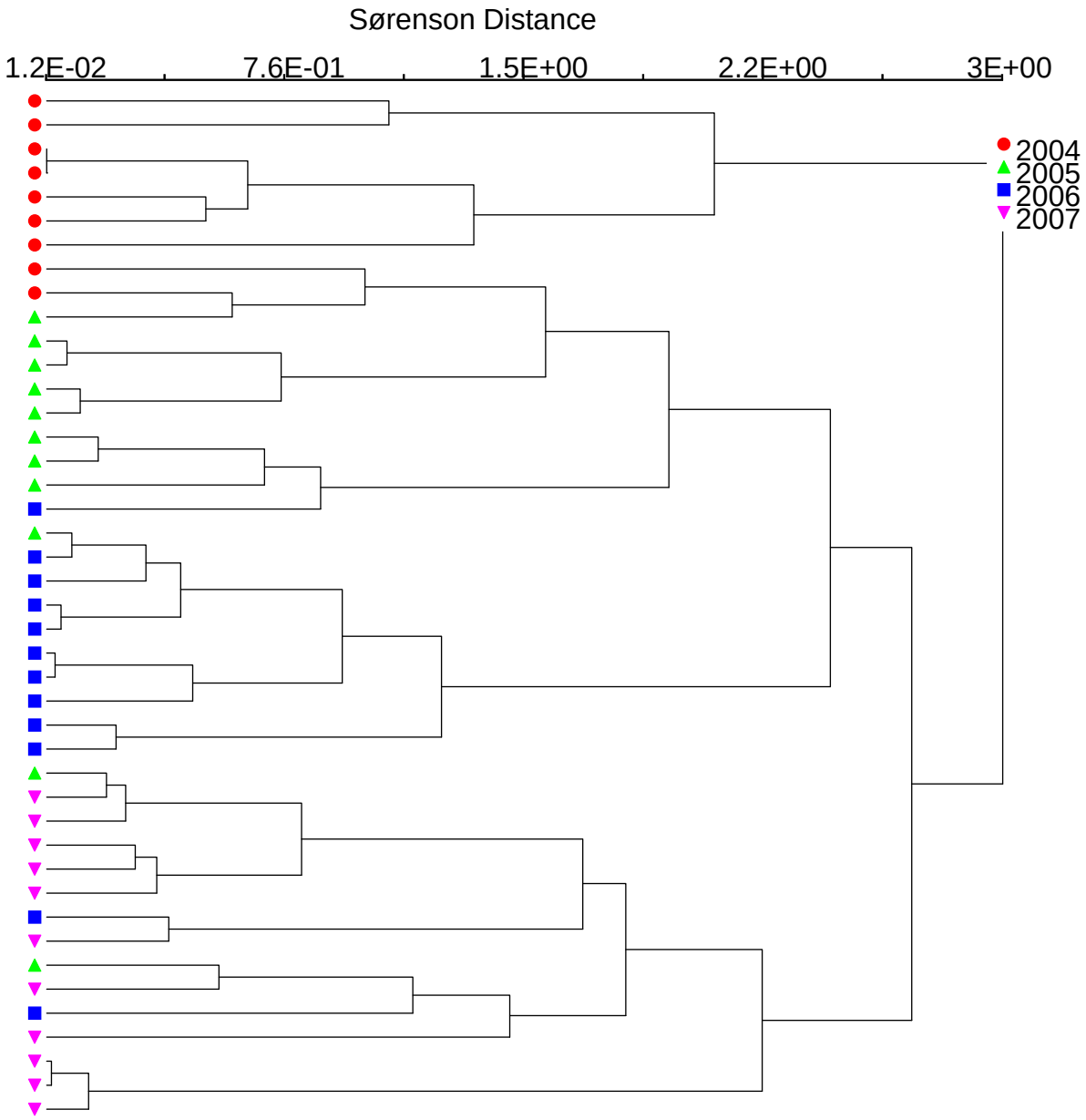


Figure 3.80. Hierarchical clustering dendrogram of all bacterial T-RLP profiles from years 2004-2007

Three identified outliers (> 2.5 standard deviations) were removed from analysis.

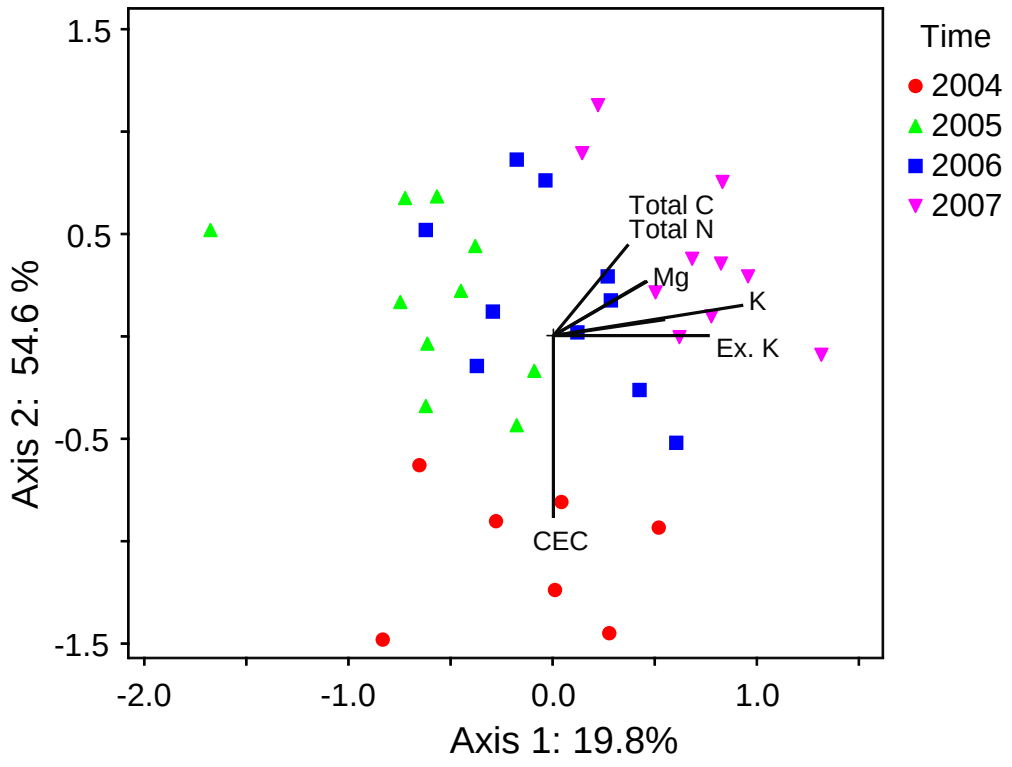


Figure 3.81. NMS ordination of bacterial profiles from years 2004-2007.

The percent variation shown on each axis is shown in parentheses. Below are the r^2 values for correlations with the environmental data. All properties with $r^2 \geq 0.2$ are shown.

	r^2
Mehlich K	0.529
Mehlich Mg	0.314
Total Carbon	0.261
CEC	0.509
Exchangable K	0.437

Chapter 4. Evaluating the effects of soil microbial properties on plant gene expression

Introduction

This chapter describes two experiments conducted in addition to the four year vegetable rotation. As discussed in the literature review, there is not much known about the soil microbial community in regards to how it is affected by soil management and in turn, how that might affect crop productivity. Various methods of evaluating plant gene expression have been used to study the effects of a range of stresses and environmental conditions on plant growth. These methods could also be used to determine if changes in the soil microbial environment alter plant gene expression in order to test the hypothesis that high microbial biodiversity and activity have a positive influence on the growth of plants. The first experiment discussed in this chapter uses microarray technology to measure plant gene expression in soybean grown under conventional and organic management. The second experiment was designed to use real time RT-PCR to analyze gene expression in tomatoes grown in the green house in sterile and biologically active soil. This experiment was not completed due to constraints on time and funding, but is included for the purpose of showing the progression of experimental design in attempts to get to the essence of these fundamental questions. Both of these experiments were designed to provide preliminary data that could be used to secure funding for more thorough and detailed experiments.

Evaluating changes in gene expression of soybean (*Glycine max*) grown under organic and conventional systems

This experiment investigated the use of microarray technology to determine if management induced soil chemical and biological properties can affect gene expression in plants. A main characteristic of organic agriculture systems is an emphasis on soil improvement techniques such as the use of cover crops,

compost, and other soil amendments. One objective of these practices is to encourage and maintain the activity and diversity of soil microbial populations, which are responsible for many vital soil processes including the decomposition of organic matter, nutrient cycling and stabilization of soil structure (Sylvia, 1998). Several studies have reported differences in soil chemical and biological properties, including increased soil microbial diversity and activity, in soil under organic management as compared to conventional (Clark et al., 1998; Øvreås et al., 1998; Shannon et al., 2002; Bending et al., 2004; Cardelli et al., 2004; Crecchio et al., 2004; Monokrousos et al., 2006; Esperschütz et al., 2007; Fließbach et al., 2007; Wu et al., 2008). There is conjecture that a more active and diverse soil microbial community contributes to the productivity and sustainability of a system by improving plant resistance to pest and disease and promoting better nutrient uptake efficiency (Altieri, 1999; Barrios, 2007; Brussaard et al., 2007). However, given the limitations of what is known about soil microbial populations and ecosystem dynamics, insight into the full relationship between soil biodiversity and plant health continues to be elusive.

A vast majority of soil microorganisms cannot be cultured and studied in the laboratory (Ward et al., 1990). Nonetheless, important discoveries have been made regarding soil microbe/plant relationships. It has been shown that bacteria engage in an exchange of chemical signals known as quorum sensing. These chemicals affect gene expression and are essential in pathogenic and symbiotic associations with plants (Bauer and Mathesius, 2004). Furthermore, plants can respond to the presence of some bacterial chemical signals and even excrete similar chemicals that influence bacterial behavior (Mathesius et al., 2003). Agriculturally significant quorum sensing activities have been discovered in the rhizosphere relating to plant health. For example, the bacteria *Serratia liquefaciens* MG1 and *Pseudomonas putida* IsoF have been shown to colonize the roots of tomato plants and increase the plant's systemic resistance to the fungal leaf pathogen, *Alternaria alternata*, by the production of the quorum sensing chemical N-acyl-L-homosterine (Schuhegger et al., 2006). A similar

relationship between the bacteria *Pseudomonas fluorescens* 2P24 and suppression of wheat take-all (*Gaeumannomyces graminis var. tritici*), a fungal disease that causes root and crown rot, was found (Wei and Zhang, 2006).

While the direct study of the soil microbial community as a whole remains a challenge, analysis of plant gene expression has potential to illuminate how soil biodiversity can affect plant growth. For example, if greater soil biodiversity improves a plant's ability to resist attacks from pests and diseases, then this should be apparent in the up-regulation of defense response genes, such as a study that found non-pathogenic, "bio-control" bacteria were able to stimulate a systemic defense response in *Arabidopsis* (Pieterse et al., 1996). A recent study finding gene expression differences in tomato plants grown with either black plastic or hairy vetch mulch bolsters the idea that management induced soil properties can elicit genotypic responses in plants (Kumar et al., 2004).

Several options for measuring gene expression are available, however microarray technology has the advantage of rapid, genome-wide assessment of thousands of transcripts simultaneously (Schaffer et al., 2000). A microarray gene chip such as those manufactured by Affymetrix (Santa Clara, CA) contains a high-density arrangement of gene-specific oligo-nucleotides that can be hybridized to fluorescently labeled mRNA extracted from tissue samples; the amount of hybridization is then quantified by a high-resolution scanner (Schaffer et al., 2000). The quality and reproducibility of manufactured high-density micro-arrays have been extensively reviewed, and the technology is increasingly being used to investigate stress responses in plants (Eisen and Brown, 1999; Schaffer et al., 2000; Wullschleger and Difazio, 2003). The Microarray Core Facility at the University of Kentucky provides the service of analyzing gene chips manufactured by Affymetrix, making it possible for researchers to conduct microarray experiments without having to incur the substantial expense of the necessary equipment. The organisms that can be investigated with microarray analysis depend on the gene sequence information available in the form of

expressed sequence tags (ESTs) (Schaffer et al., 2000). Of the plant gene chips available from Affymetrix, soybean was chosen as the most appropriate for this study due to its agricultural importance and available resources. The soybean gene chip contains approximately 37,500 transcripts of *Glycine max* (www.affymetrix.com/support/technical/datasheets/soybean_datasheet.pdf).

Several known microorganisms have been shown to influence plant responses to stresses such as disease, and with a majority of soil community remaining uncharacterized, it is extremely likely that other types of plant/microbe interactions are taking place. There is mounting evidence to suggest that alternative management practices such as those practiced in organic systems can alter soil chemical and biological properties, resulting in a more diverse and active soil microbial community; however there is no direct conclusive link proving exactly how this affects plant health. More research in this area is needed in order to better understand the sustainability of management practices that affect the soil microbial community. This experiment compared organic and conventional management systems to address the question of whether plant gene expression is affected by different soil chemical and microbial properties.

Materials and methods

Experimental design

In August of 2005, two plots on the University of Kentucky Horticultural Research Farm were chosen to grow soybean under organic and conventional management. The organic area had been under cover crop for the last 3 years with rye/vetch, buckwheat, and fescue, respectively. The land where this area is located is part of 4.5 hectares reserved for organic research – it had been free of any unapproved inputs for 6 years at the time of planting. The conventional plots were in a field which has been under continuous conventional management, including mold-board plowing and applications of chemical fertilizers, herbicides,

fungicides, and pesticides. The field was planted in melons the previous year and had been fallow with no cover-crop since the fall of 2004.

The conventional and organic areas were split into three 3069 m^2 subplots. The organic plots were tilled using a spader (Imant, Reusel, Holland) and 60.5 kg/ha of compost was added and disked in. Cultivation was the sole method of weed control. The conventional plot was tilled with a mold-board plow and disk harrow. The pre-plant incorporated herbicide Dual II Magnum was applied at 1.5 l/ha according to label instructions. The locally available, non-GMO soybean variety HT-381-STS (Southern States Cooperative Inc., Richmond, VA) was inoculated with *Rhizobium japonicum* and planted both plots at a rate of 112 kg/ha, as recommended by the University of Kentucky extension publication for soybean cultivation (Herbek et al., 1988).

Soil sampling

In order to determine soil differences between the two areas and variability within each area, a composite of twenty soil sample cores at depths of 0-5 cm and 4-10 cm were taken from each subplot. In order to minimize contamination between subplots, latex gloves were worn during sampling and soil probes were rinsed with water and then 95% ethanol between the sampling of each subplot. The composite of soil cores was thoroughly mixed inside sample bags by gloved hand. A sub-sample of soil from these composites was sent to the UK Regulatory Services to be analyzed for basic nutrient content, organic matter, base saturation, and water holding capacity, and another subsample was used for the analysis of basal respiration. The remainders of the soil composites were frozen inside the sample bags at -80 C for microbial DNA analysis pending the results of the microarray.

Soil microbial activity

Basal respiration of soil samples was measured as an indication of soil microbial activity. Approximately 2 grams of field moist soil were added to 160 ml serum bottles, which were then capped, sealed and kept in the dark at 25°C. At days 2, 9, 16 and 23, a 100 µl sample of the headspace was removed with a syringe and the CO^2 content was measured by a Shimatzu GC-8A gas chromatograph (Shimatzu Scientific Instruments, Columbia, MD) (Smith, 1983).

Plant tissue sampling

Plant tissue sampling was done at flowering, before leaves started to senesce. Both treatments had visually identical stands of soybean. Any plant that was damaged, diseased, or stunted was not selected for sampling. A no. 149 steel arch punch (C. S. Osborne & Co., Harrison, NJ) was washed thoroughly and baked at 150˚ C for four hours. The arch punch was used to take a sample from the soybean leaf approximately 2.5 cm in diameter that weighed on average 0.09 grams. The punch was cleaned with 90% ethanol, Rnase ZAP (Ambion Inc., Austin, Texas), and sterile de-ionized water between sub-plots. Four plants from each subplot were sampled, giving three pooled samples from each treatment for a total of six samples. The four leaf tissue circles were placed in a sterile 10 ml polypropylene tube with forceps (baked at 150˚ C for four hours) that were rinsed with Rnase free water and Rnase ZAP between each use. After collecting four samples, the tubes were immediately submerged in liquid nitrogen for freezing in the field and later transferred to a -80˚ C freezer.

RNA extraction

RNA extraction was performed using the TRIzol protocol (Invitrogen Corp., Carlsbad, Calif). The appropriate amount of TRIzol (according to weight of sample in grams) was added to the sample while still frozen in the tube. A ploytron tissue homogenizer was used to homogenize tissue in the TRIzol buffer, and the subsequent extraction steps were carried out. The sample was further

purified using the QIAGEN RNeasy prep kit (QIAGEN Inc., Valencia, Calif) as recommended by the UK Microarray facility and kept at -20˚C. The RNA was visualized by gel electrophoresis and quantified using a mass spectrometer – the samples ranged from 0.1 to 0.5 µg/µl. The samples were then transferred to the University of Kentucky Microarray Core facility for analysis.

Micro-array analysis

Micro-array analysis with the Affymetrix soybean gene chip was performed by the University of Kentucky Microarray Core Facility (www.mc.uky.edu/UKmicroarray). Before analysis, a quality assessment of RNA samples is performed on an Agilent 2100 Bioanalyzer (Agilent Technolgies, Santa Clara, CA), which generates an RNA integrity number (RIN).

Results

Soil analysis

The soils of the two plots were determined to be different according to the results of basal respiration as an indication of microbial activity, and chemical properties. Basal respiration was demonstrably higher in the organic section than the conventional (Figures 4.1, 4.2). At 0-5 cm, the mean CO2 increase over 21 days in the organic soil was 379.61 µg CO2 g-1 dry weight soil, compared to 34.50 in the conventional. At 5-10 cm, the organic soil increased 74.15 µg CO2 g-1 dry weight soil compared to 43.98 in the conventional soil. Soil chemical properties that were significantly different ($p < 0.05$) in ANOVA are listed in Table 4.1. Mehlich III extracted calcium in the organic plots were 4776 kg ha-1, around 1877 more than the conventional. The organic plots were in the range of 9-10 kg Zn ha-1 and 7-9 cmol kg-1 exchangeable calcium, while the conventional plots had levels of 3-5 kg Zn ha-1 and 5-6 cmol kg-1 exchangeable calcium. Base saturation was around 17% higher in the organic. The

conventional plots were significantly more acidic, with a pH of 5.92 compared to 6.87 in the organic plots.

Microarray

Data analysis of the soybean gene chip was performed by the statisticians at the Microarray Core Facility. Normalization (Mas5 as recommended by Affymetrix) and quality control procedures were carried out. Probesets with all absent readings were removed from further analysis (a total of 24,531), leaving 36,504 probesets. Each probeset was fit to a model using a Proc T-test (SAS v9.1) with $\alpha = 0.01$ significance level to determine if there was a significant difference between the mean expression of the two treatment groups. Of the probesets tested, 432 were significantly different, which is not sufficient to conclude an overall significant result. An estimated 96.41% of genes analyzed did not change due to the treatment; while a small number of individual genes may indeed be differentially expressed, the sheer number of probesets analyzed means that smaller differences can not be determined statistically.

Discussion

No significant gene expression differences were found between the organic and conventional treatments, which were shown to have different soil chemical and microbial properties. In retrospect, several difficulties with the treatment groups and the use of microarray in this type of experiment were apparent.

While soil chemical properties and microbial activity (as measured by basal respiration) were significantly different between the two treatments, it is possible that differences were not enough to elicit a gene expression response from the soybean plants. The differences seen in soil properties could be attributed to a number of factors. The conventional plots had been continuously cultivated in recent years, therefore lower soil nutrient levels and microbial activity would be

expected. The organic plots had been fallow and in fescue or cover crops in recent years. Despite the differences, soil properties in both treatments were adequate for soybean growth, and both plots produced vigorous, visually identical stands. At the time of this study, the only gene chip available from Affymetrix for an agricultural crop that could feasibly be used for this experiment was for soybean. Soybean grows easily with little management when there are no major disease problems, and does not require many inputs. This provided little opportunity for management differences between the two treatments. Additionally, microarray technology may be too broad of a tool – if there are expression differences among only a small amount of genes then it is statistically impossible to find them when looking at tens of thousands of genes at once. It was suggested by the statistical analysts at the UK Microarray Core Facility to examine the 432 significant genes for the types of differences that would support the original hypothesis, such as defense or stress genes (annotation for each gene was provided on the spreadsheet). The literature could be checked for any studies which might support a particular gene as representing a true difference between treatments, such as the differentially expressed genes found in the study by Kumar et al. (2004), in which compared tomatoes grown in black plastic or hairy vetch mulch. This was done, but no promising information could be found.

Microarray is frequently employed in experiments where varying degrees of stress are being imposed in test subjects, which often leads to significant amounts of genes being affected (Cushman and Bohnert, 2000; Eckardt, 2008). In this experiment, both stands of soybeans were visually identically healthy, with no major insect or pest damage. It is possible that in the absence of stress, different soil conditions or management practices do not affect the gene expression of healthy plants. Alternately, the soils may not have been different enough with respect to the microbial community.

Conclusions

While it cannot be concluded from this experiment that gene expression in plants can be affected by management practices or the soil differences observed, the premise that the soil microbial community can affect the growth and health of plants in ways that have not yet been discovered remains an intriguing challenge. With what is known about agriculturally (and economically) important plant microbe associations, and what is not known about the soil microbial community in general, suggests that there is more to be discovered that could impact agricultural productivity and the search for sustainable solutions to the challenges of future food production. What has been learned from this experiment has lead to a more focused approach to the problem of investigating a largely unknown ecosystem with no direct way to observe its functioning. The following experiment was designed to investigate specifically the effects of microorganisms on plant gene expression by attempting to compare plants grown in sterilized field soil to plants grown in native, biologically active field soil. Due to time and funding constraints, this experiment was not completed.

Evaluating the effect of soil biodiversity on gene expression in tomato plants

As expressed in previous chapters, there is a need for further knowledge of the soil microbial ecosystem, how it interacts with plants, and how those interactions can impact the sustainability of different land uses and management practices. Research currently underway in sustainable agriculture is making progress in optimizing techniques for organic and reduced chemical input systems as well as characterizing changes in soil quality under these systems (Bending, 2004; Liebig, 1999). What is missing is the actual correlation between the effects these practices have on soil biodiversity and the effects on the overall health and success of the plant. Some research has been done to address the possibilities of such endeavors. There is discussion of the potential for uncovering the

pathways of specific signals between bacteria and eukaryotic organisms that could induce defense and/or symbiotic responses in plants and animals (Ramamoorthy et al., 2001; Bauer et al., 2005; Compant et al., 2005). Research using cDNA cloning techniques have shown differences in expression of certain genes and proteins that function in disease defense and senescence in tomatoes grown under conventional and alternative management (Kumar, 2004).

Because of the difficulties of attempting to study unknown and unculturable soil microorganisms, it can be more useful to study the soil microbial ecosystem as one entity. This experiment proposed to examine the gene expression of tomato plants grown in soil in the presence and absence of microorganisms. There are several methods for determination of differential gene expression, including representational difference analysis (RDA) and microarray analysis. However, these methods are expensive, and in the case of RDA, time consuming. Preliminary data that demonstrates the ability to detect gene expression differences in these treatments is needed before committing the time and funding for these analyses. Real-time reverse transcriptase PCR, or quantitative RT-PCR measures the level of specific mRNA transcripts in a total RNA sample (Nolan *et al.*, 2006). It was decided to use select genes from the study done by Kumar et al., in which differential gene expression was found in tomato plants grown on black plastic and hairy vetch mulch (Kumar et al., 2004). While soil microbial properties were not explored in this study, in other research fatty acid profiles of the soil microbial community was shown to be significantly different in these two systems (Carrera et al., 2007). Genes chosen from the results of the Kumar study provide a reasonable starting point for examining the effects of soil microbiology on tomato gene expression. It is proposed that at least five genes should be selected for quantitative real-time RT-PCR analysis of the two treatments: Nitrate reductase (*NiR*), glucose-6-phosphate dehydrogenase (*G6PD*), osmotin, cytokinin-responsive gene (CKR), gibberillic acid 20 oxidase (*GA20-oxidase*), and the chaperone gene *Bip*. These represent genes in nitrogen response, fungal defense, hormone response, and stress response.

Materials and Methods

Experimental design

Sixteen plants were grown in soil that has been sterilized by autoclave, and 16 plants were grown in soil that has been autoclaved, but re-inoculated with native soil. Leaf tissue samples were collected after 6 weeks and frozen at -80°C for RNA extraction. RNA samples from 4 plants were pooled in order to reduce biological variability, for a total of 8 samples: 4 from the sterile treatment and 4 from the non-sterile treatment. The experiment was replicated simultaneously using added fertilizer, as an observation comparison and insurance in the event that fertility in autoclaved soils too low for the plants to grow well.

Soil Collection and Preparation

Soil was taken from a tilled field in the organic research area of the Horticulture Research Farm, up to a depth of 15 cm. Soil was passed through a 4 mm sieve to homogenize and mixed with perlite (2:1 ratio). Perlite was used to facilitate drainage and prevent the soil from caking in the pots. It is inert and autoclavable.

Soil/perlite mix was placed in shallow glass pans to a depth of 2.5 cm. The soil was moistened and pre-incubated for 2-3 days before autoclaving to stimulate microbial growth. The pans were covered with aluminum foil and autoclaved at 0.10 Mpa and 121 ˚C for 1 hour. Pans were left to sit undisturbed for several days, allowing any spores that survived the first autoclave to become active so that they could be killed in a second autoclave (Wolf and Skipper, 1994). Preliminary trials testing the basal respiration of autoclaved soil showed that complete sterilization of the soil does not occur with this method. Some microorganisms form survival structures that can withstand multiple autoclaving, and can then flourish without competition upon a return to favorable conditions. More complete sterilization can been achieved using gamma irradiation,

however this was not an option for this experiment (Wolf et al., 1994). Because the microbial community would be fundamentally altered by autoclaving, it was ultimately decided that inoculation with field soil should serve to introduce sufficient diversity for comparison. Frozen soil samples can be used to more fully characterize the soil microbial diversity in each treatment by T-RFLP or similar means. To ensure that autoclaving did not cause extreme changes in soil properties that would interfere with plant growth, samples of autoclaved soil and inoculated autoclaved soil were sent to the University of Kentucky Regulatory Services lab for analysis of chemical properties (see Chapter 1, Materials and Methods, Soil chemical properties). While autoclaving does cause changes in some soil properties, it is generally regarded as an appropriate for studies involving re-inoculation and plant growth (Wolf et al., 1994).

After two autoclave cycles, half of the soil was removed from the pans and placed into 16 pots that were surface sterilized in 5% bleach solution. The remaining sterilized soil was inoculated by adding 5% (w/w) native soil and mixing thoroughly before placing into pots (Shaw et al., 1999). Soil samples were taken from each pot for basal respiration analysis at planting and when plant tissue was harvested. Approximately two grams of soil was placed in 160 ml serum bottles, which were then sealed and incubated in the dark at 25° C. A 100 ml sample of the headspace in the serum bottle was removed with a syringe and analyzed on a Shimatzu GC-8A gas chromatograph (Shimatzu Scientific Instruments, Columbia, MD) (Smith, 1983).

Tomato Plants

Big Boy hybrid tomato plant seeds (Fayette Seeds, Lexingon, KY) were surface sterilized by soaking in a 5% bleach solution for five minutes followed by rinsing with sterile water before being planted in the pots using autoclaved utensils. Pots were covered with plastic wrap to decrease air contamination; a small hole was made for the seedlings to grow through.

Plants were watered individually by a sterile 25 ml pipette with either autoclaved water or autoclaved fertilizer solution (18-18-21) made with Miracle-Gro water soluble tomato plant food (Scotts Miracle-Gro Products Inc., Marysville, OH). Plants were grown in a greenhouse for 4 weeks, at which time it was determined that sufficient foliar growth for tissue sampling was achieved.

Plant tissue sampling

One leaf was sampled from each plant (from the third node down from the apex). Four leaves, one from each plant in the replicate, were combined in one sterile 10 ml polypropylene tube and flash frozen in liquid nitrogen and stored at -80 C.

Real-time PCR

At this time, mRNA has not yet been extracted from frozen plant tissues. The intended course of action is to grind the frozen leaf tissue in liquid nitrogen, then extract mRNA using the Qiagen RNeasy Plant Mini Kit (Valencia, CA) and quantify using nano-cell (Thermo Scientific) . Quantitative RT-PCR will be performed using primers for the genes listed in Table 4.3 (Nolan et al., 2006).

Results

Soil analysis

Regulatory Services analysis determined the pH of the autoclaved and inoculated soil samples to be 6.5. An increase in Mehlich III extracted and exchangeable P, Mg and Ca was observed in autoclaved soil, but no indication that major changes in soil chemical properties had occurred. Basal respiration from soil samples collected at planting and at the time of tissue collection were compared according to the amount of increase in µg CO_2 detected in the headspace of the serum bottles over one week. Means were compared with ANOVA. At the time of tissue sampling, the average change in CO_2 was

statistically higher in the inoculated treatment, indicating increased microbiological activity during plant growth (Table 3.2).

Tomato plant growth

Plants grew well in both fertilized and non-fertilized treatments. At the time of tissue sampling, it was observed that among the between the unfertilized treatments, the inoculated plants appeared taller. Plant heights were measured, and indeed a significant difference was found (Table 3.2). This difference was also observed in the fertilized treatments.

Summary

At this time, RNA samples from plant leaf tissue have not yet been analyzed. Much work still needs to be done to optimize the RT-qPCR, confirm specificity of the primers and validate the control gene. The observation of a phenotypic response between the treatments encourages the hypothesis of a gene expression difference. It is possible that microbial activity could have provided additional mineral nitrogen from decomposition of organic matter in the soil, or facilitated nutrient uptake. Leaf tissue samples from fertilized and un-fertilized treatments, along with root tissue samples and soil samples from each of the pots at planting and at tissue harvest remain frozen at -80°C.

This experiment was conducted primarily as means to gather preliminary data and to test the design for studying plants grown in sterilized field soil in the greenhouse. So far, it was shown that autoclaved field soil, sieved and mixed with perlite, was an appropriate media for plant growth in small pots in the greenhouse. Sufficient drainage was achieved and the plants grew vigorously. Achieving complete sterilization is problem – gamma irradiation appears to be better method, but is not readily available to most laboratories. Other methods that achieve total sterilization are not compatible with re-inoculation or plant growth (Wolf et al., 1994).

If the results of RT-qPCR do show a difference in gene expression between these two treatments, it is possible that funding for more in depth experiments could be obtained. If no differences are found using the five genes selected for comparison, then the next approach would be conduct a full investigation of gene expression by RDA or similar technique. While valid reasons were presented for the use of these particular genes in this experiment, it is entirely possible that they are not among the genes differentially expressed in the conditions that were created. It would also be useful to add plants grown under the same conditions that were stressed by disease or insect damage.

This experiment has the potential to contribute information towards the challenges of studying sustainability in agricultural systems as it relates to soil quality and its effects on plant health. Uncovering the consequences and pathways of plant/microbial interactions is the next step forward in understanding the soil agro-ecosystem and how it can be sustainably managed.

Table 4.1. Soil chemical properties that differed between conventional and organic plots.

		Organic	Conventional
0-5 cm	Mehlich III Ca (kg ha^{-1})	4771.57 (983.99)	2894.84 (253.88)
	Mehlich III Zn (kg ha^{-1})	10.864 (1.24)	4.67 (1.53)
	Ex. Ca (cmol kg^{-1})	8.63 (1.37)	5.74 (0.55)
	% Base saturation	69.20 (7.93)	52.27 (5.08)
	Soluble salts (dS m^{-1})	0.13 (0.04)	0.06 (0.01)
	pH	6.87 (0.29)	5.92 (0.06)
5-10 cm	Mehlich III Zn (kg ha^{-1})	10.61 (2.10)	4.51 (0.91)
	Ex. Mg (cmol kg^{-1})	0.70 (0.14)	1.07 (0.15)

Statistical significance determined at the $\alpha = 0.05$ level. Standard deviations are shown in parenthesis.

Table 4.2. Basal Respiration at planting and at the time of tissue sampling, and plant height.

	Autoclaved soil	Autoclaved soil + inoculation with field soil
BR at planting (µg CO_2 g-1 dw soil)	103.33 (63.49)	129.45 (32.08)
BR at tissue sampling (µg CO_2 g-1 dw soil)	72.28 (27.84)*	101.47 (31.71)*
Plant height at tissue sampling (cm) unfertilized	20.42 (2.42)*	22.78 (2.60)*
Plant height at tissue sampling (cm) fertilized	22.19*	24*

Values are means of samples taken from the 16 pots in each treatment and represent the increase in µg CO_2 detected after one week of incubation in sealed serum bottles by gas chromatography. Standard deviations are shown in parentheses.
* means are statistically different at $\alpha = 0.05$ level

Table 4.3. Proposed target genes and tentative primers for RT-qPCR

Gene	GenBank	Forward primer	Reverse primer
NiR	BG791272	TGGCCGGGAGGAAGGATAC	GCTCGTCCATGTCATCTGCT
G6PD	AW035603	TTTGCTTCCCACAAGATTTCTC	GCTAGGTGGAGCCACTACAG
CKR	AW218786	GCCAGGGTGTTTGGTGGTTTG	GGTGCTGGTAGAGGTTCATGTC
GA20-oxidase	AI774919	GTCGAACTGTGATGGAGGTACAAAG	CCCACACTTGAGGCATTTCTC
Bip	AF049898	TGGCGTTCCATCAGTCCAAA	CCGCTCTGTGTAGGCAACTTT

Genes listed in this table were found to be differentially expressed in tomato leaf tissue when plants were grown using either hairy-vetch or back plastic mulch (Kumar et al., 2004).

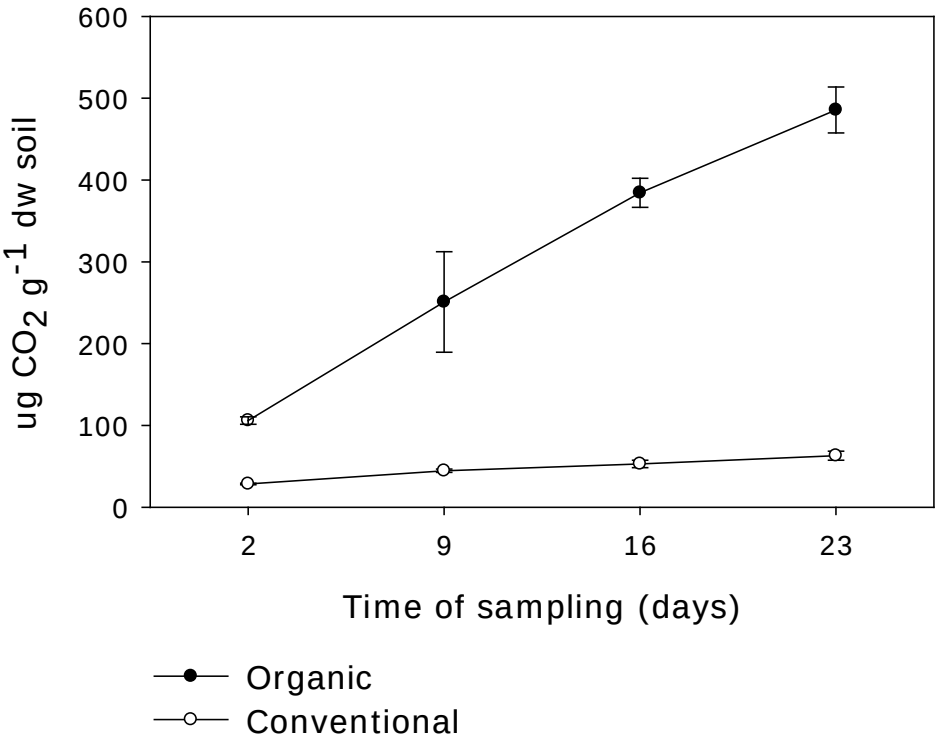


Figure 4.1. Basal Respiration of organic and conventional plots before planting, at 0-5 cm soil depth.

Data points are averages of the three sub-plots

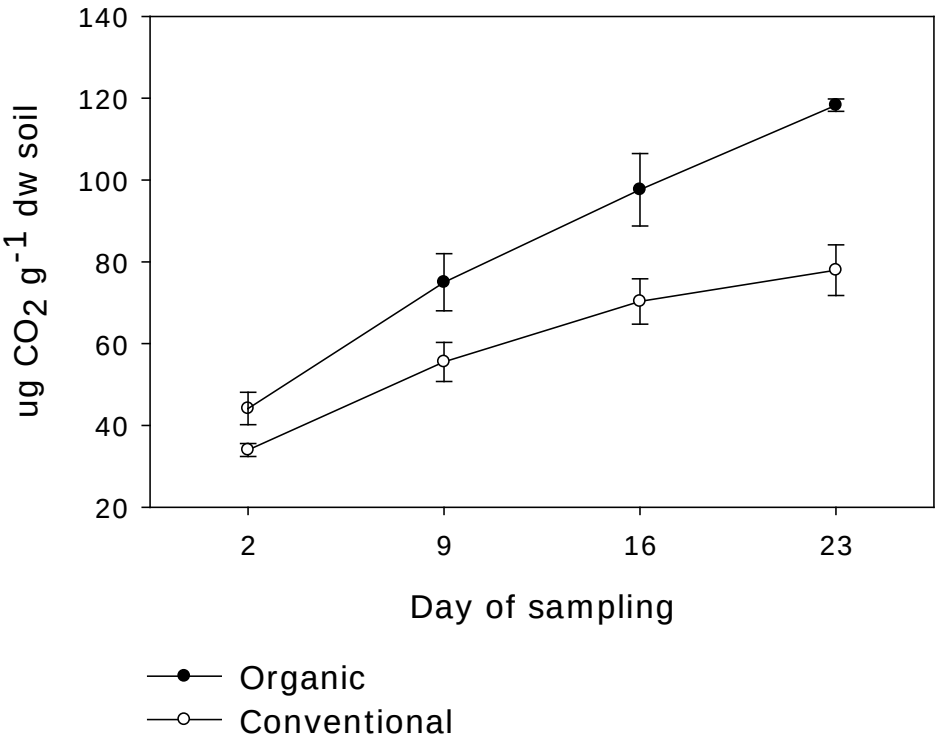


Figure 4.2. Basal Respiration of organic and conventional plots (averages) before planting, at 5-10 cm soil depth.
Data points are averages of the three sub-plots

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